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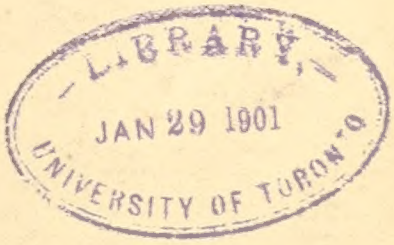


MEDICAL
DEPARTMENT

REPORT OF THE
LABORATORY OF PATHOLOGY
NUMBER ONE
1900

REPORT

OF THE

LABORATORY OF PATHOLOGY

OF THE

UNIVERSITY OF BUFFALO

MEDICAL DEPARTMENT.

NUMBER ONE.

1900

THIS report consists chiefly of reprints of papers that have already been published in medical journals. The article on Fat-Necrosis is the only exception, and particular thanks are due to Dr. Charles G. Stockton for permission to use papers of which he was one of the joint authors in the preparation of that article.

The expenses of publication have been defrayed by the Permanent Faculty of the Medical Department.

HERBERT U WILLIAMS,

Director of the Pathological Laboratory.

November 1, 1900.

CONTENTS.

[Reprint from the Buffalo Medical Journal, November, 1900.]

An Inquiry Into the Existence of Autochthonous Malaria in Buffalo and its Environs.[1]

PRELIMINARY REPORT ON SPECIES OF MOSQUITOES AND BLOOD EXAMINATIONS.

By IRVING P. LYON, M.D., and ALFRED B. WRIGHT, Stud. Med., Buffalo, N.Y.

[From the Pathological Laboratory of the University of Buffalo.]

THE investigations here recorded were suggested by two chief considerations, viz., first, the importance of accurately ascertaining the species of mosquitoes found in this locality with respect to establishing whether or not the *Anopheles* exists here, and, second, also, the frequence of the diagnosis of malaria by general practitioners of medicine.

A careful consideration of the first point seems to be one of the most feasible and scientific methods of throwing light on the second point, for it is now a demonstrated fact that various species of mosquitoes embraced within the genus *Anopheles* are the direct agents of the dissemination of malarial disease, and moreover the cumulative evidence of the more recent studies of this subject has focused strongly on the probability, that the *Anopheles* is the only active agent involved in the inoculation or production, by any means, of malaria.

We have, therefore, approached the investigation along two principal lines of inquiry, as follows: (1) What species of mosquitoes are found in and about Buffalo? (2) What evidence of the existence

1. Read before the Buffalo Academy of Medicine, October 16, 1900.

of autochthonous malaria here is furnished by examination of the blood?

Before presenting the facts which we have gleaned by pursuing these lines of inquiry, we wish to make clear that we recognize the occurrence of occasional cases of malaria here, in which the infection had been previously acquired at places far distant from Buffalo. We have, therefore, excluded from consideration all cases of malaria in persons who gave a history of recent previous malarial infection and in those who had traveled at a distance within a period of a few weeks previous to the development of malaria at this place. We have also excluded all malarial cases in which the history was not explicit and positive with respect to these points.

Report on the Species of Mosquitoes collected in and about Buffalo.—Our investigation of the species of mosquitoes found in this vicinity covers the period May 12—October 7, 1900. During this time we have examined 374 mosquitoes caught within and near the city of Buffalo, chiefly from points along Lake Erie and Niagara River. The infrequence of mosquitoes in this region and the difficulty of obtaining them, in spite of our unremitting efforts, explain why we have not a larger number of specimens upon which to base our report. For convenience of reference we have compiled the following table, showing the date and place of capture, and the number, species and sex of the mosquitoes.

TABLE OF OBSERVATIONS.

Date of Capture.	Place.	Genus.	Species.	Sex.	No.
May 12, 1900	*191 Barthel street	Culex	Stimulans	Female	2
" 13, "	Rose Hill, Canada	"	{ " / Impiger	{ " / "	2 / 1
" 26, "	Foster's Flats, Can. (near whirlpool)	"	{ Stimulans / Impiger	{ " / "	9 / 5
" 27, "	*22 Park street	"	Stimulans	"	2
" 27, "	Marilla, N. Y.	"	"	"	1
" 29, "	Alden, N. Y.	"	{ " / Impiger	{ " / "	2 / 2
" 29, "	*Park Lake	"	Stimulans	"	2
" 30, "	*South Park, near lake	"	{ " / Impiger	{ " / "	3 / 5
" 30, "	East Concord, N. Y., swamp	"	{ " / Stimulans	{ " / "	12 / 16
June 2, 1900	*221 North street	"	"	"	1
" 7, "	" " "	"	"	"	1
" 8, "	Gardenville, N. Y.	"	{ " / Impiger	{ " / "	3 / 4
" 9, "	Ebenezer, N. Y.	"	{ " / Stimulans	{ " / "	3 / 1
" 10, "	West Seneca, N. Y.	"	"	"	3
" 15, "	Grand Island, N. Y.	"	{ " / Impiger	{ " / "	16 / 25
" 21, "	Clarence Center, N. Y.	"	Stimulans	"	1
" 25, "	*221 North street	"	"	"	1
" 25, "	*Park Meadow	"	"	"	3

Date of Capture.	Place.	Genus.	Species.	Sex.	No.
June 28, 1900	*110 High street	Culex.	Impiger	Female	1
July 2, 1900	*191 Barthel street..	"	"	"	1
" 3, "	*221 North street.....	"	Stimulans	"	1
" 19, "	*O'Neil's Park	"	"	"	1
" 19, "	*O'Neil's Park, woods near	"	"	"	9
			Impiger	"	94
" 19, "	Point Abino, Canada	"	"	"	23
			Stimulans	"	15
" 22, "	*Squaw Island	"	"	"	18
			Impiger....	"	6
" 23, "	Fort Erie, Canada, woods near	"	"	"	15
			Stimulans	"	12
			"	"	5
August 4, 1900	Athol Springs, N. Y.	"	"	Male	4
			Impiger....	"	12
				Female .	5
" 16, "	*52 Park street	"	Stimulans...	"	4
" 19, "	*122 Grape street.....	"	"	"	1
" 19, "	228 Goundry street, Tonawanda, N.Y.	"	"	"	2
			Impiger	"	4
September 2, 1900...	Tonawanda, N. Y., swamp	"	"	"	27
			Stimulans	"	10
" 6, "	*191 Barthel street	"	Impiger	"	3
" 8, "	*379 William street.....	"	"	"	7
			Stimulans	"	6
" 14, "	*191 Barthel street ..	"	"	"	6
			Impiger.,..	"	12
" 17, "	*Cazeu via Park .	"	Stimulans...	"	4
" 25, "	Niagara Falls, N. Y.	"	"	"	1
October 2, 1900.....	*800 Main street .	"	"	"	1
			"	"	3
" 5, "	*77 High street	"	†Pungens	Male	1
" 7, "	Athol Springs, N. Y..	"	Stimulans	Female .	2
			Impiger	"	2

* Indicates points within the city of Buffalo.

† The wings of this specimen were injured so that the identification of its species, as *C. pungens*, by Dr. L. O. Howard, was pronounced "probable." As the *C. pungens* and *C. impiger* are very similar, this specimen may have been, perhaps, *C. impiger*, especially as no other specimen of *C. pungens* was found in our collection, whereas *C. impiger* constituted more than half of our specimens.

It will be seen by reference to this table of observations that the 374 mosquitoes were caught on thirty-nine different occasions, from thirty-one different places, of which fifteen were points within and sixteen points outside of the city of Buffalo. Many specimens were obtained from lowlands, marshes, ponds, and streams, chiefly in the region of Lake Erie and Niagara River, in fact from places to which public report attached a reputation of being malarious. The specimens were obtained by months as follows: May, 64; June, 63; July, 117; August, 37; September, 84; and October, 9. The observations were therefore made during six consecutive months, representing the "malaria season", and the distribution during these months was fairly uniform, with the exception of the last month, October. From points within the city, 121 mosquitoes were obtained, and 253 from places outside of Buffalo.

Of the 374 specimens, every mosquito belonged to the genus, *Culex*, and not a single example of *Anopheles*, the malaria-bearing

variety, was found. The distribution by species and sex is shown as follows:

[illegible]	193	Male 12 Female 181	
[illegible]	180	Male 4 Female 176	Male 17 Female 357
[illegible]	1	Male 1	
Total	374	374	374

It is thus shown that *C. impiger* and *C. stimulans* were about equally distributed, and that a third species, *C. pungens*, was represented by only a single specimen, the identification of which was only probable, due to the injury to its wings.

The unequal sex-distribution will also be noted. Only seventeen males, as opposed to 357 females, were found, and curiously also all of them, excepting one, *C. pungens*, at a single time and place. We are acquainted with no other statistics showing the sex distribution of adult mosquitoes in nature. The apparent rarity of males may find its explanation in the fact that males are not blood-suckers and, therefore, would not be so frequently found as females near people, where they would be caught.

In identifying the species we were assisted by Mr. E. P. Van Duzee, librarian of the Grosvenor Library, and by Dr. L. O. Howard, entomologist to the Division of Entomology, U. S. Department of Agriculture, Washington, D.C., to whom we wish to here record an expression of our appreciation and indebtedness for their very kind assistance. In collecting the mosquitoes we have received assistance from many interested and kind friends, of whom our especial thanks are due to Dr. and Mrs. DeLancey Rochester, Dr. Eugene A. Smith, Mr. Albert Frey, and Mr. T. F. Ellis. Our especial indebtedness and warmest thanks are due to Dr. Herbert U. Williams, professor of pathology, University of Buffalo, in whose laboratory the work was conducted, for his active co-operation and generous assistance for many months in this study.

To the courtesy of Mr. Charles A. Bentz, student of medicine in the University of Buffalo, we are indebted for the accompanying diagrammatic map of the region of Buffalo and its vicinity, from which we collected the mosquitoes for this study. The locations from which mosquitoes were caught are represented on the map by points in red.

In concluding the report of this part of our investigation, we may summarize the findings by the statement that the known malaria bear-


QUEENSTON
LEWISTON
NIAGARA COUNTY
LOCKPORT
ROYALTON CENTRE
St DAVIDS
NIAGARA RIVER
LEWISTON
CAMBRIA
SANBORN
ROYALTON
WALMORE
NIAGARA
WHEATFIELD
PENDLETON
NIAGARA FALLS
LASALLE
BERGHOLTZ
St Johnsburg
ERIE CANAL
TONAWANDA CREEK
COUNTY LINE
NIAGARA FALLS
CHIPPEWA
CANADA
GRAND ISLAND
NORTH TONAWANDA
TONAWANDA
MARTINSVILLE
WOLCOTTVILLE
CLARENCE
NEWSTEAD
GETZVILLE
ISWORMSVILLE
CLARENCE CENTRE
AMHERST
WILLIAMSVILLE
EGGERTSVILLE
SNYDER
ELLICOTT CREEK
BOWMANSVILLE
SQUAW I
BLACK ROCK
SCAJAQUADA Cr
BUFFALO
CHEEKTOWAGA
LANCASTER
ALDEN
RIDGEWAY
ROSE HILL
FORTERIE
Pt Abino
LAKE ERIE
BUFFALO CREEK
ELMA
MARILLA
WEST SENECA
SPRINGBROOK
EAST ELMA
POTTERSVILLE
WALES CENTRE
EAST AURORA
ATHOL SPRINGS
ABBOTTS CORNERS
EAST HAMBURG
AURORA
WALES
HAMBURG
EDLEWOOD
EIGHTEEN MILE CREEK
SOUTH WALES
EDEN VALLEY
LITTLE SISTERS CREEK
HOLLAND
EVANS
EDEN
BOSTON
COLDEN
ANGOLA
BIG SISTERS CREEK
GLENWOOD
FARNHAM
BRANT
NORTH COLLINS
SARDINIA
EAST CONCORD
CONCORD
ERIE COUNTY

ing genus of mosquito, *Anopheles*, was not found among 374 specimens, and its absence we regard as strong evidence against the development of malaria here.

Report on the Blood Examination of Cases of Suspected Malaria.—At a meeting of the Buffalo Academy of Medicine, in June, 1900, the writers, with the coöperation of Dr. Herbert U. Williams, offered their services gratis for making blood examinations in search of the malarial parasites for any physician who suspected the existence of malarial disease in a patient. In addition to this offer we also put ourselves in communication with the authorities and staffs of all the larger hospitals and requested the opportunity of examining the blood of all suspected malarial cases. As a result of these tenders we have examined during the past summer the blood of twenty-eight cases, five being in hospitals and twenty-three the private patients of practicing physicians. *In every case, without exception, the blood was found negative for malarial organisms.* Indeed, during the entire summer we have found malarial parasites in the blood of only one case, referred to us by Dr. Grover W. Wende, that of a man who had recently arrived in Buffalo after extended travels.

The Experience of Other Observers with Blood Examinations.—We have solicited evidence also on the existence of autochthonous malaria in this locality from the experience of various physicians who have been in the habit of making blood examinations, as consultants, whose reports are briefly summarized, as follows:

Dr. Herbert U. Williams.—Has never seen a case of autochthonous malaria in Buffalo, and does not believe it occurs here.

Dr. Herman G. Matzinger.—During the past five years has never seen a case of malaria that originated here.

Dr. Albert E. Woehnert.—Has seen only a few cases of malaria in Buffalo in several years, and no case that he could affirm originated here.

Dr. Thomas B. Carpenter.—Has never seen a case originate in Buffalo.

Dr. George Roberts.—Has seen no cases here.

Dr. Earl Lothrop.—During the last five years has examined the blood of about 250 cases of suspected malaria, and found parasites in only three, all of whom came from malarious places distant from Buffalo.

Dr. N. G. Russell.—Has never seen a case of malaria that originated in this locality.

Dr. Charles S. Jewett.—Is of the opinion that autochthonous malaria exists in the city; has found malarial parasites (tertian) in only one case which he thought acquired the infection in the city, but of this he is not certain, having made no special inquiries at the time of examination.

Dr. Julius Ullmann.—Has never seen a case originate in Buffalo.

Dr. Vertner Kenerson.—Has seen only one case that he suspected of local origin, but he made no special inquiries on this point.

We may add our own testimony to the above statements, to the effect that during the past two years one of us (Lyon) has made numerous blood examinations and has found malarial parasites in only four cases, all of whom acquired the infection at places far distant from Buffalo.

The unanimity of the evidence contained in the above statements against the autochthonous development of malarial infection in and about Buffalo—for many examinations were made, especially in the hospitals, on patients from contiguous towns—needs no comment.

We have also solicited the opinions of many physicians from all sections of Buffalo and from neighboring towns, who have not made blood examinations, as to the existence of malarial infection originating in this locality. The responses have varied in every degree from a positive affirmation to a flat denial of its origin here. Those who affirmed its occurrence offered no evidence in support of their opinion, save the clinical and therapeutic behavior of their cases. We may be permitted to comment in this connection that opinion, based alone on clinical and therapeutic observations, can claim only slight consideration as opposed to the scientific and demonstrable evidence of the microscope.

Inquiries at all the large hospitals as to the frequence of malarial cases, without reference to the source of origin of the infection, elicited the general reply that such cases were only rarely seen.

We may, therefore, summarize this part also of our inquiry with the statement that we have been unable to discover any evidence that proves the development of malaria in and about Buffalo in recent years.

Before closing this paper we wish to call attention to a few further points bearing on the subject.

Geology and Topography.—Our observations have been made in the city of Buffalo and in neighboring towns within a radius of twenty-five miles, the great majority of observations having been made in places close to Lake Erie or the Niagara River within or very close

to the city of Buffalo. Buffalo is situated at the eastern end of Lake Erie, whose elevation above sea-level is 573 feet. The city rises above this level in general from 20 to 100 feet. The county of Erie and the surrounding district from which our observations were taken range from about 500 feet above sea-level on the north at Niagara Falls to about 1,500 feet in the hills in the southern part of Erie county. In general the district is underlain by a rock-bottom, composed chiefly of limestone or shale, and is covered with clay or clay and sand or gravel to a varying depth. The district is well drained by numerous streams and contains few swamps and lowlands. Niagara River, the outlet of Lake Erie, starting at Buffalo, flows northward through the district with a strong and rapid current. The district is only sparsely wooded, and is chiefly an agricultural country.

Climate.—The climate of the region is salubrious. The mortality records of Buffalo show one of the lowest death-rates recorded for any city in the United States. The prevailing winds are from the southwest, passing over Lake Erie before reaching Buffalo. The influence of the lake tends to produce an equable temperature at all seasons. The summer temperature is relatively low, the winter not severe. Humidity is increased by the influence of Lake Erie. The city and district are swept by frequent winds, blowing off the lake, sometimes of violent character. The weather of the past summer is shown by the United States Weather Bureau records to have been above the average in temperature, humidity and precipitation.

It is therefore seen that the geology, topography and climate of the region are all opposed to the expectation of finding malaria endemic here. The country is high, dry, cool, windy and sparsely wooded.

Special effort was made to study cases of suspected malaria and collect mosquitoes from places that are commonly called malarious, viz., Tonawanda,* Squaw Island, South Buffalo, and the swamps, ponds, and streams of the region, and many observations were made from these places, but always with negative results for the presence of malaria or the *Anopheles*.

It is well known that the *Anopheles* does not inhabit the interior of towns and is found in general only in rural districts near natural collections of water, such as ponds and streams, where it breeds. Thus, in notoriously malarious regions, such as the Roman Campagna and the Chesapeake Bay region, the *Anopheles* has not been found within the cities of these regions, viz., Rome and Baltimore, nor do

*

cases of malaria develop autochthonously within these cities, except sporadically. We did not therefore, a priori, expect to find *Anopheles* and autochthonous malaria within the city of Buffalo, and only, if at all, in the outside rural districts, and our special efforts were directed to obtaining evidence from outside places.

Inquiries at the Pan-American Exposition grounds, just outside of the park, where the soil has been upturned and artificial waterways have been in construction during the past summer, have shown that no cases of malaria have been reported among the workingmen employed there. We have several times attempted to find mosquitoes there, but always unsuccessfully.

The weather of the past summer has been more than usually favorable to the spread of mosquitoes. The average temperature, humidity and rainfall have been above the average. Still, mosquitoes have not been frequent and have been difficult to obtain, and the clinical diagnosis of malaria has probably not been more frequent than in previous years.

As previously stated, occasional cases of genuine malaria have been seen in Buffalo, imported from a distance. The return of our regiments from the South after the Spanish-American war, in 1898, brought back many cases of the disease. Had the *Anopheles* been a common inhabitant of this region, it is fair to assume that these imported cases of malaria would have afforded opportunity for the infection of the *Anopheles* and the subsequent development of an outbreak of malaria. This, however, did not occur. On the other hand, it is possible to suppose that the *Anopheles* might be imported to this region from a distance and become resident. In reply to the suggestion of this possibility, we need only say that we have failed to find it, and, too, in a season favorable to mosquitoes. Still we do not deny that *Anopheles* may have abounded here in the past or may do so in the future.

That mosquitoes, and even the *Anopheles*, may exceptionally be carried to a distance from their breeding-places is evidenced by the observation of Grassi, who observed a few specimens of *Anopheles* in a railway carriage traveling from Florence to Berlin. We have ourselves during the past week caught a mosquito (*Culex*) in a railway car on the Lehigh Valley Railroad, near Geneva, New York, in traveling from Buffalo. It may be that by such agencies and others the spread of *Anopheles* into new localities is effected, and in case the other conditions necessary are present, sporadic cases of malaria may arise. Such sporadic cases, due to such exceptional circumstances, would

not however alter the general fact that malaria appears to be rare and is not endemic here. It seems to be a generally accepted fact that genuine malaria was in earlier years common throughout this region and through the northern and eastern states, although at present it is a rare condition away from the seacoast and in the country north of New Jersey.

The distance to which mosquitoes may be carried exceptionally by natural agencies is unknown. The available evidence tends strongly to show that mosquitoes usually remain close to their breeding-places and do not migrate far. They resist being carried by winds and storms and seek shelter against the violence of the elements. However, to what distance they may exceptionally be carried by violent storms is only conjectural.

We have had a secondary purpose in view in pursuing these investigations, namely, to contribute our minute share of evidence to the world-wide study that is now in progress of the relation of the distribution of the various species of mosquitoes to the production of malaria. All contributions, however, fragmentary, isolated and apparently insignificant, form an integral part of the foundation of evidence upon which the future knowledge of malaria-distribution must rest.

In conclusion we wish to state that we make no claim to having settled the question of the occurrence of autochthonous malaria here, but we believe that only by such studies as we have made can reliable data be obtained on which to base an opinion. It is sincerely hoped that further contributions to the subject will make certain what our work has made only very probable.

531 Franklin Street

NOTE.—Since the publication of the paper of Lyon and Wright a few additional notes have been obtained.

Mr. E. P. Van Duzee reports having caught between twenty and thirty mosquitoes at Hamburg, May 20 and 26, 1900, and a smaller number at Buffalo Plains, June 19, 1900. All were of the genus Culex.

Twenty mosquitoes secured during August, 1900, in the wards of the Erie County Hospital at night, have been examined. All were of the genus Culex.

Mr. M. F. Adams, a local entomologist, states that he has been collecting and identifying mosquitoes in Buffalo and on Grand Island during the summer of 1900. Between 300 and 400 individuals were examined. These proved to belong to the genus Culex, with three exceptions, which were of the genus Anopheles. Two of the latter were found in a garden on Summer Street June 9th, the third on Norwood Ave. August 7th. Unfortunately the specimens of Mr. Adams have been lost, so that his important observation cannot be verified.

Cover-glass preparations of blood from a case of suspected malaria occurring in Tonawanda were sent to the laboratory about the middle of November, 1900. The malarial parasite could not be found. The same result followed the examination of preparations from a case in the city November 21, 1900.

EDITOR.

[Reprinted from JOURNAL OF CUTANEOUS AND GENITO-URINARY DISEASES, November, 1898.]

POROKERATOSIS, WITH REPORT OF CASE.[1]

BY GROVER WILLIAM WENDE, M.D.,
Clinical Professor of Dermatology, University of Buffalo.

IN the year 1887, Domenico Manocchi, an Italian, put on record a case presenting a singular appearance of the skin, which he thought to be a form of ichthyosis hystrix. After a lapse of six years, the same case was brought to the notice of Vittorio Mibelli, also an Italian, who pronounced the lesion peculiar and exceptional, described the pathological process as distinctive, and characterized the affection as porokeratosis. Simultaneously with the observations made by Mibelli, there appeared in the *Giornale Italiano delle malattie veneree e della Pelle* an article by Respighi, describing a number of cases, which, in a clinical and pathological sense, were the same as those already reported by Mibelli. One year later Respighi classified his particular cases under the name of hyperkeratosis eccentrica. In 1896, M. B. Hutchins of Atlanta, Georgia, published the clinical details of a case of porokeratosis occurring outside of Italy, he having had the patient under observation since 1892, one year prior to Mibelli's announcement. In 1897, T. C. Gilchrist of Baltimore, Md., made reports of eleven cases which proved that the affection had followed a course of transmission from grandmother to grandchildren. In June of the same year, Dr. Max Joseph gave a graphic and exhaustive account of two cases encountered by him in Germany.

The pathological alterations incident to this disease are apparently non-inflammatory in character and are confined to the epithelial layer of the skin, which, at the outset, appears as a specialized horny patch that soon becomes depressed in the center, afterward developing into a seam (Hutchins) or dyke (Mibelli). The periphery of the lesion reveals a gradual development, is unique in character, and cannot justly be likened to any other cutaneous manifestation. A pronounced line, which may be either uninterrupted or broken, marks its course. This, at times, is studded with miliary projections forming in the outline of the skin involved.

The lesion presents a configuration that may be either regular,

[1] From the Pathological Laboratory of the University of Buffalo.

wavy, polycircular, or grotesque, and that varies in size from a single centimeter to dimensions sufficient to cover the entire forearm. It shows a predilection for exposed surfaces, especially the hands and face—sometimes the feet, rarely the body. When the hands and feet are affected, the manifestation is usually in close proximity to the joints, the nails becoming secondarily involved. Occasionally the buccal mucous membrane is found to be the seat of the disease. The skin within the inclosure produced by the peculiar seam may be apparently normal, or atrophic and slightly depressed, with loss of hair. Complete anidrosis has also been observed in these areas, undoubtedly due to the destruction of the sudoriparous apparatus. Small oval concretions, isolated or grouped, resembling the miliary projections described in the seam, are sometimes discovered on the surface of the affected parts. The disease is found to attack both sexes, but, thus far, judging from the cases reported, the male sex predominates. Nor is there any discrimination with regard to age, the disease occurring alike in childhood, adolescence, and maturity. Spontaneous retrogression, or actual disappearance, has never occurred except in one of the cases published by Mibelli. After existing for a number of years, the disease gradually progresses and continues through life. Subjective symptoms may or may not be present.

Proof of hereditary transmissibility is often evident, as shown by the cases published by Gilchrist, where eleven members of one family were declared to be affected in the course of four generations. The results obtained from a microscopical investigation by Mibelli demonstrate the fact that the process, from its commencement, is practically a hyperkeratinization. The alteration is first seen to take place in the epithelial lining of the tubules of the coil glands, as a cell proliferation or acanthosis. Subsequently, there is manifest a plugging due to marked hyperkeratosis, followed by atrophy, which extends to and includes the various layers of the skin as well as the sebaceous glands and hair-follicles.

A case occurring in my own practice which may be regarded as typical, although not as extensive in its distribution as some already recorded, nevertheless confirms the details of the original description of the disease. My observations were supplemented by a few experiments. The history of the case follows.

On the 17th of September, 1897, at the dispensary of the University of Buffalo, I first came in contact with the patient, a woman, who, at the time, was forty-five years of age, strong and healthy, with the exception of the present annoying trouble. Except an attack of small pox, which occurred at the age of 20, the previous history was negative

The patient is of American birth, intelligent, married, and pursues an active and industrious life. Her father was ninety-seven years old at the time of his death, and her mother is now eighty years of age. She has a brother and a sister, both of whom are in good health. As far as known, no member of the family, near or remote, was ever affected with a like malady. Her complexion is that of a brunette; the skin is thick and pigmented; the hair is hazel, and the mucous membrane of the mouth, as far as visible, is rose red.

It was soon discovered that the expression of the eruption with which the patient was affected was striking and exceptional. She was, therefore, promptly sent to a photographer who secured the accompanying picture. It was impossible from the appearance of the lesion and the symptoms present, to make a positive diagnosis. Consequently, there was prescribed a 20-per-cent. ointment of mercury, to be spread on cloth and held *in situ* by means of adhesive plaster. It was also thought that benefit might be received from a mixed treatment. K. I. and Hg. were, therefore, given internally. That these remedies were deserving of an extended trial was judged from the knowledge possessed at the time regarding the etiology and pathology of the disease. The patient did not return to the dispensary for three months. The remedies, however, were employed unremittingly, but with no positive effect, except that the local itching was somewhat subdued.

After reading Hutchins' article, which appeared on page 373 in the JOURNAL OF CUTANEOUS AND GENITO-URINARY DISEASES, October, 1896, the syphilitic theory was dropped and the mercury application abandoned, as it was found by a comparison of the case in hand with his, that the patient also exhibited unequivocal marks of porokeratosis. As to the peculiar way in which the trouble began, the patient stated that, five years ago, she became aware of the existence of a small, rough, scaly papule, situated upon the dorsal side of the interdigital fold, between the thumb and the index-finger of the left hand, which speedily assumed the appearance of a new growth, was about the size of a pea, and resembled a seed wart. For a long time its dimensions were unchanged, causing considerable annoyance, as, upon the slightest provocation, the lesion would become excoriated. This hypertrophy, with its occasional denudations and peculiar sensitiveness, prevailed for more than one year, when it suddenly assumed quite a different character, being replaced by a scaly ring about the size of a coriander seed, with a marked border, and a steady and gradual inclination to develop and spread peripherally. The irritation and consequent torment were materially lessened upon the subsidence of the excrescence, but increased as the circle becam larger.

For the past two years the condition has been accompanied by a sudden, colorless, evanescent tumefaction of the skin surrounding the lesion. Different localities of the forearm, even at points remote from the lesion, were, at times, affected. Its development has always been sudden and rapid, invariably culminating in less than two hours and vanishing with equal rapidity. This condition would frequently manifest itself after the patient retired for the night and would disappear before morning. The swelling always affected the same hand and arm, but not always the same region. Another peculiarity complained of was the intensity of the itching whenever the hand came in contact with water, especially if it was warm; in fact any change of temperature aggravated the affection. On December 15, 1897, two weeks after all local and internal treatment had been discontinued, a most careful examination of the case was made, which disclosed no evidence of organic or functional disturbance of the heart, lungs, or any of the abdominal organs. An analysis showed the urine to be normal. The peripheral and central nervous system, as well as the organs of special sense, exhibited no unhealthy condition.

Following the examination, a few days later, the circumscribed cutaneous edema, already referred to, was observed. The intermittent lesion, measuring two inches in diameter, occupied the anterior surface of the forearm; the color of the skin was natural; and, by the next day, the swelling had wholly disappeared, with the exception of two small scaly pea-sized patches, thought to indicate the inceptive stage of the disease. The forearm presented a normal aspect. The scaly spots were not examined microscopically. The principal lesion, presenting the same feature as those described by Mibelli and Respighi, was found on the hand of the same arm, at the base of the thumb and index-finger, and encroaching upon the interdigital fold. The patch represented an area measuring sixty millimeters at its longest diameter and thirty-five at its shortest. It was quadrilateral in shape and clearly defined by the unusual continuous zig-zag border which constitutes the pathological characteristic of the disease. This abnormal boundary between the patch and the healthy skin was especially interesting from the fact that it was raised into a pronounced ridge, horny and unyielding, having a marked central rift which varied in depth. The ridge measured two millimeters in both width and height. At the points where the prominence was most flattened the rift was entirely obliterated. The lesion, in color, resembled the normal skin, although somewhat heightened by the deposit of extraneous matter. Apart from the ridge, the skin was not apparently influenced by the morbid process, but that confined within the area and contiguous to the inner edge of

the boundary line varied from a slight pink to a glossy white—the latter being more toward the center. The entire inclosure was slightly depressed, with a loss of hair-follicles. The natural furrows of the epidermis were somewhat less apparent, as shown in the photograph.

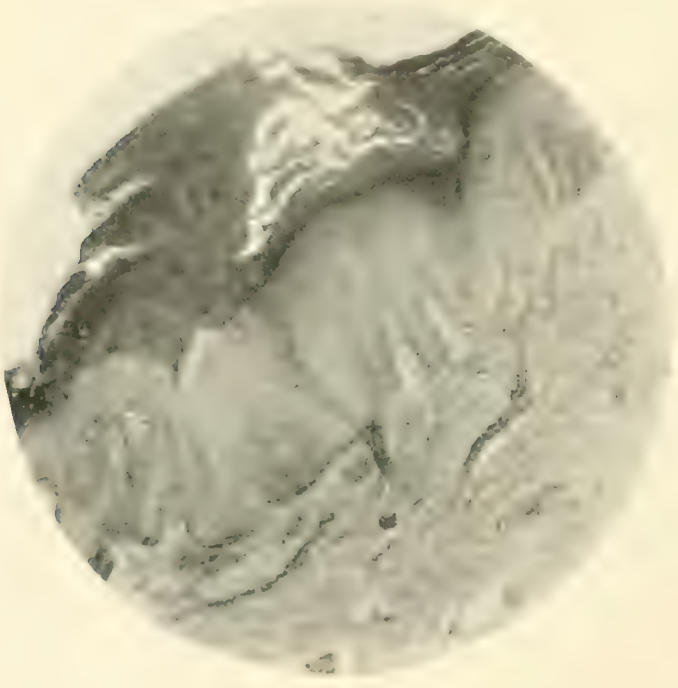
A deep spot of cicatricial tissue, resembling parchment, was especially noticeable. A single hair, abnormally bent, was found within the inclosure. At the first examination, prior to the mercurial application, numerous horny nodules, the size of a rape-seed, were observed in close proximity to the inner portion of the ridge, on the wrist side, which could easily be removed by the finger-nail, leaving minute indentations. Numerous conical elevations, analogous to the small concretions embedded in the plaque, existed in the rift, some of which were scattered, others combined in groups. These may readily be detected by viewing the photograph (Fig. 1) with a magnifying glass.

The affected area also displayed an invariable anhydrosis and esteatosis of the surface. The internal administration of pilocarpine practically illustrated a total suppression of perspiration in the inclosure, whereas an increased flow was everywhere apparent, even in close proximity to the ridge. The application of mercurial plaster for the first three months simply retarded the growth of the lesion, while the more recent employment of the curette and the use of salicylic acid temporarily removed the rim. However, it was readily re-formed and, after a lapse of four weeks increased to the dimensions of six millimeters.

In the five fragments of skin, which were removed for microscopical examinations, from the border of the parts affected, the histopathological appearances characteristic of the disease were clearly manifest. After the usual hardening in alcohol and embedding in celloidin, vertical sections were cut and stained. One description will suffice for the different methods which were employed in the various microscopical examinations. In the sections examined, wherever the ridge existed, the epidermal layer was greatly augmented. Within the inclosure, in portions of the stratum corneum, there was a general hyperkeratosis. There was also hypertrophy of the rete Malpighi. A marked hypertrophy was manifest in portions of the layers previously affected. In other portions, it was discovered that the disease principally affected the stratum corneum. This developed into a ridge extending above the normal level of the epidermis, which was easily removed by means of a curette. A vertical section of this unusual elevation, after having been subjected to a picrocarmine stain, was found to consist of an aggregation of epithelial cells, arranged, with more or less regularity, into either perpendicular or oblique columns. The nuclei of some of these cells were deeply colored, others were wholly unaffected. Near the surface

where the cells were most irregular, they were partially destroyed and the remnants were combined into groups; while those in certain localities, and contiguous to the stratum mucosum, were rounded and often presented distinct nuclei. The rete nearest the rift was usually thickened and its interpapillary prolongations were greatly exaggerated. The cylindrical cells of the basal layer were well preserved; the same was true of those comprising the prickle layer, when not in close proximity to the rift, in which event they assumed an appearance of roundness and were usually widely separated. The stratum lucidum

FIG. 1.



was only rarely perceptible; it was often ill-defined, more frequently invisible. The stratum granulosum, without exception, revealed a marked hypertrophy, apparently consisting of five to eight layers of granular cells, which was especially augmented in the vicinity of the sweat-ducts, and which took the picrocarmine and hematoxylin stain exceedingly well. This pronounced alteration in the granular layer has led me to believe that possibly it may be the seat of the origin of the disease.

The superficial blood-vessels in the papillar and retecular layers

of the corium, under the long papillæ, where the process was most active, were dilated and contained an increased number of leucocytes. This change was especially apparent in the vicinity of the sweat-glands. The morbid alterations in the glands themselves and in their ducts were conspicuous. The sweat-pores within the inclosure were plugged with numerous horny cells. Their lining was seldom smooth and their orifices were but slightly involved. However, in the ridge, the orifices of the ducts presented a superimposed mass of horny cells, extending as far down as the rete and producing, in many instances, a terminal plug. In the ridge itself, where the process was most active, the sweat-apparatus exhibited no evidence of atrophy. On the other hand, within the circle formed by the ridge, the lumen of the ducts was often diminished in caliber—sometimes destroyed.

The glandular tissue comprising the sebaceous glands was noticeably diminished in those parts where the changes were active.

At times, the accumulation of epithelium would be found in the mouth of the sebaceous follicles, while but little of the sebaceous glands remained recognizable. In the ridges the hair and its follicles could not be discovered at all.

The effort to determine the real source of this exceptional affection is attended by many difficulties, for the reason that, as yet, our knowledge of it is so limited that we can only approximate definite classification, being governed by its anatomical aspects as pointed out by Mibelli. We can never know what is primary and what is secondary, or what occasions the accumulation of horny cells in the duct of the coil-glands, and their plugging, until we possess a rational appreciation of the etiology of the disease.

Respighi conjectures that the trouble may be of a parasitic nature, and endeavors to discover its origin by staining for micro-organisms and by experiments in transplantation. Bacteriological examinations were also made by him, and various culture-media were used, but no evidence of the existence of micro-organisms resulted. Prior to the reading of Respighi's article, the writer made some thirty inoculations upon four different individuals—ten on December 17, 1897, ten on January 1st, and ten more on January 15, 1898. The method of procedure consisted simply in a slight scarification of the parts to be inoculated, sufficient, however, to produce an oozing of lymph, when a small amount of the pulverized horny substance from the rift of the lesion heretofore described was gently rubbed into the locality scarified, allowed to dry, and protected from friction by means of an ordinary round corn-plaster. The scarifications, with the exception of a few upon the arms, were mostly made upon exposed parts. But a single

one of the thirty inoculations mentioned seemingly proved a success—this occurred upon the unaffected hand of the patient having the disease, after ten unsuccessful attempts. All the others revealed the ordinary crusting, which readily healed and came to nothing. The first indication of successful inoculation was the appearance of a small, rough, non-inflammatory spot, which, within ten days, gave evidence of a slight elevation in the center, and extended peripherally until it attained the size of a rape-seed. It then began to flatten, without any visible signs of either discoloration or inflammation. Two weeks later it was accompanied by intense itching, and the patient, fearing a difficulty like the one from which she had so long suffered, became uneasy and demanded its immediate removal. The operation was delayed as long as possible; however, after the expiration of ten weeks, to obviate further anxiety on the part of the sufferer, it was performed.

The extirpated tissues were then subjected to a microscopical examination which, in the main, revealed features not unlike those found in sections taken from the typical lesion. The similarity consisted in the pathological changes associated with the orifices of the coil-glands and the presence of a horny cell proliferation within their ducts. Near the center of the section of the orifice of a gland was clearly perceptible. This was sufficiently dilated to form a funnel-shaped opening, plugged with a mass of horny cells that projected to the extent of one millimeter beyond the normal level of the epidermis. The tissues surrounding the projection were loosely arranged and greatly increased in thickness. Hypertrophy of the mucosum was also evident. Apart from this there was nothing suspicious.

In my opinion, had the manifestation been allowed to remain, it would undoubtedly have produced the disease, with all the clinical features fully developed. A large number of sections from the newly formed lesion were stained for micro-organisms, after the methods of Gram, Gram-Weigert, and with Ziehl's carbolfuchsin, but without result. These same methods were used in connection with the original lesion, but no micro-organisms were demonstrated.

Bibliography.

1. Mibelli, Vittorio, "An Uncommon Form of Keratodermia, Porokeratosis." "International Atlas of Rare Skin Diseases," No. 9, 1893.

2. Mibelli, Vittorio, "Contributo allo studio della ipercheratosi dei canali sudoriferi," *Giornale Italiano della malattie veneree e della pelle*, Fasc. iii. 1893.

3. Mibelli, Vittorio, "Beitrag zum studium der Hyperkeratosen der

Knauedrusengange (Porokeratosis)," *Monatshefte für praktische Dermatologie,* Band. xvii, 1893.

4. Respighi, Mmilio, "Di una ipercheratosi non ancora descritta," *Giornale Italiano della malattie veneree e della pelle,* Fasc. iii, 1893.

5. Tommasoli, Pierleone, "Sulla nuova ipercheratosi recentemente studiata dal Professor Mibelli (porokeratosis) e dal Dott. Respighi." (Pseudo-lichen circinato porocheratosico)." *Commentario clinico delle malattie cutanee* ecc., anno ii, N. I., 1804.

6. Mibelli, Vittorio, "Uber die porokeratose," *Monatshefte für praktische Dermatologie,"* Band xx, No. 6.

7. Mibelli, Vittorio, "Ipercheratosi Eccentrica," *Giornale Italiano della malattie veneree e della pelle,* Fasc. ii, 1895.

8. Respighi, Emilio, "Di Una Singolare Ipercheratosi," *Giornale Italiano della malattie veneree e della pelle,* Fasc. ii, 1896.

9. Reisner, Alois, "Ein Fall von Porokeratosis," Inaugural Dissertation, Strasburg, 1896.

10. Hutchins, M. B., "A Case of Porokeratosis (Mibelli)," JOURNAL OF CUTANEOUS AND GENITO-URINARY DISEASES," vol. xiv, October, 1896.

11. Joseph Max, "Porokeratosis," *Archiv. für Dermatologie und Syphilis,* Band. xxix, Hft. 3, June, 1897.

12. Gilchrist, T. Casper, "A Case of Porokeratosis (Mibelli), or Hyperkeratosis Eccentrica (Respighi), with a Remarkable Family History," *Bulletin of the Johns Hopkins Hospital,* vol. viii, No. 74, May, 1897.

13. Ducrey and Respighi, "The Localization of Porokeratosis upon the Buccal Mucous Membrane," *Annales de Dermatologie et de Syphiligraphie,* No. 1, 1898.

[Reprint from Buffalo Medical Journal, October 1,

THE RELATION OF THE SPECIFIC GRAVITY OF THE BLOOD TO ITS PERCENTAGE OF HEMOGLOBIN.

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SINCE the publication of our paper in the *Medical News* of December, 1895,[1] we have added a number of new cases to our list of observations. In addition to these, we have included in the present paper much of the description of methods given in our former paper, together with the results of our first observations.

For the determination of the hemoglobin in our first series we used v. Fleischl's hemometer and Gowers's hemoglobinometer. We made no count of the blood corpuscles, in comparison, but it has been found by Jones, Hammerschlag, Schmaltz and others, that the specific gravity corresponds more closely with the amount of hemoglobin than with the number of red blood-corpuscles.

For determining the specific gravity of the blood, a variety of methods have been used. A good method, but one which takes considerable time, is that with the pycnometer. This, as used by Schmaltz, is a capillary tube of 0.1 cc.m capacity, which is first weighed empty, then when filled with water, and, finally, when filled with blood. The weight of the tube is subtracted and the specific gravity calculated from the weight of the blood as compared with that of the water.

Fano, in 1882, used a method which depends upon the principle that a body when immersed in a fluid will float indifferently in that fluid when the specific gravities of the two are the same. The fluid used, was a solution of gum in water. To make this heavier, he

1. Busch and Kerr: "The Relation Between the Specific Gravity of the Blood and its Hemoglobin Percentage." *Medical News*, December 21, 1895.

added a denser gum solution. To make it lighter he added water. Blood was introduced into this and the liquid made to correspond in specific gravity to that of the blood. The specific gravity of the liquid was then determined and thus at the same time that of the blood.

Roy, in 1884, devised a method applying this same principle. He used solutions of salt or other suitable substances ranging in specific gravity from 1.035 to 1.075. For making the test he used a modified hypodermic syringe. This had a small steel tube prolonged inward from the tip so as to be seen through the glass sides of the barrel. The syringe was filled with a salt solution and a drop of blood drawn into it. As it emerged from the steel tube into the solution he noted whether it rose or sank. If it sank, a new solution of higher specific gravity was chosen; if it rose, one of lower specific gravity was taken. Finally, one was obtained in which the blood neither rose nor sank, or two were found in one of which it rose and in the other sank. In the latter case the specific gravity of the blood was between the two.

Roy's method as modified by Jones, is as follows: The apparatus consists of from 20 to 25 one-ounce glass bottles filled with standard solution of glycerin and water, differing one from the other by 0.001 specific gravity and ranging from 1.027 to 1.075; a number of fine glass pipettes drawn out to a point and bent at right angles near the tip; a cylindrical glass jar of about one-dram capacity; and a number of clean suture-needles. By means of the pipette the blood is introduced into the one-dram jar filled with one of the standard solutions chosen by guess from the appearance of the patient. It is then blown out gently, being given an impetus in a horizontal direction owing to the bent tip of the pipette. If it rises or sinks other solutions are chosen, as in Roy's method.

Landois used solutions of sodium sulphate ranging between 1.050 and 1.070.

Siegel has adopted a device whereby the frequent standardisation with the Jones-Roy method is obviated. He covers the surface of his standard solutions with a layer of olive oil, introducing the blood through this by means of a glass tube covered by a rubber cap.

In our determinations we have used Hammerschlag's method. The necessary apparatus consists of a urinometer jar, a urinometer, a pipette of small caliber, a glass rod, some fine steel pens, a bottle of chloroform, one of benzol, and a mixture of the two. The finger, after being washed with bichloride and alcohol, is punctured. A good sized drop of blood is introduced into the chloroform-benzol

mixture. If the drop sinks the mixture must be made heavier by adding chloroform. If it rises, the mixture is too heavy and must be made lighter by adding benzol.

It is desirable not to divide the drop of blood into several, but on the other hand, care must be taken to get the chloroform and benzol thoroughly mixed by stiring with the glass rod. In order to obtain this end it is better, when the liquid is too heavy, to add an excess of benzol and then obtain the required density by adding chloroform, slowly and carefully until the drop of blood floats indifferently in the mixture. When this occurs, the drop of blood is of the same specific gravity as the mixture, and by determining that of the mixture with a urinometer, we have also determined that of the blood.

We have found it most convenient to obtain the blood from the middle finger of the left hand, making the puncture to the side of the tip on the palmer surface. For this purpose, we have used an ordinary sharp-pointed steel pen, with one nib broken off. The pen was sterilised by heat and a new one used for every test. The blood was drawn up into a pipette of fine caliber, which was introduced into the middle of the chloroform-benzol mixture and nearly all of the blood gently blown out. We were careful, however, not to blow all of the blood out, but to leave a little in the tip of the pipette, shaking off the adhering drop. In this way the error of mixing air with the blood was avoided.

Ziegelroth[3] claims it is better to obtain the blood from the lobe of the ear, since it is nearer the density of the blood in general.

The same chloroform-benzol mixture may be used repeatedly, if filtered after each test. The urinometer jar must be scrupulously clean; otherwise some fine particles of dust might adhere to the drop of blood and cause an error.

It is also imperative to take the reading as soon as possible after a satisfactory mixture is obtained; since there is considerable danger especially in a warm room of error through vaporisation of the volatile fluid. Besides this, the globule of blood, after a little time appears to shrink and change its shape.

The hemoglobinometer of Gowers is usually manufactured with but one colored tube which is for use with daylight only. There is another form in which there are two tubes, one for use with daylight and the other with artificial light. The one we have employed is of the former kind. In making the comparison with it, one must hold the instrument against a white background, opposite the source of light, or as recommended by Landois, between the eye and the light.

In the use of v. Fleischl's hemometer, artificial light is necessary, and the test must be made in a room from which daylight is excluded. We have for the sake of convenience adapted a simple device, allowing us to carry our dark room with us. A piece of card-board is cut so as to fit over the top of the canvas traveling bag in which we carry our instruments. The hemometer is placed in one end of the case, and a small lamp in the other. A hole is cut in the card board to give passage to the lamp chimney. Another small hole is cut so as to be directly over the well of the instrument. At the side of this hole, a window, closed by a card-board flap, is cut, for the introduction of the hand, in order to move the wedge of the instrument.

The specific gravity in health has been differently given by different observers.

MALES.		FEMALES.	
[illegible]	[illegible]	Perrier	1.051–55
Perrier	1.056–59	Davy	1,045–56
[illegible]	[illegible]	Nasse	1.055–55
[illegible]	[illegible]	Becquerel and Rodier	1,054–60
Nasse	1.055–57	Schmidt	1,056
Becquerel and Rodier	1.058–60	Quincke	1,058–60
Muschenbroeck	1.054	Jones (about)	1,055
Denis	1.059	Schmaltz	1,056
Schmidt	1.060	Becker	1,057–56
[illegible]	[illegible]	Landois	1.057–55
[illegible]	[illegible]		
Becker	1.054–60		
Landois	1.056–50		

Hammerschlag found the specific gravity of healthy males—between the age of 20 and 40 to vary from 1.057 to 1.625 and in females from 1.0535 to 1.061.

In the tropics, the average for normal adults, according to Glogner, is 1053.6, and according to Eijkman, 1.057.4.

The average specific gravity of the blood in the newborn is 1.060; for the two to four months old child it is 1057; for the twelve months old child it is 1050; and from the second to the tenth year it is 1052. Monti[4].

Jones states that venous blood is denser than arterial. Sherrington and Copeman found almost no difference in density between venous and arterial blood. In passive congestion, as in a ligatured finger, they agree that there is an immediate rise in specific gravity. This rise vanishes immediately upon the disappearance of the congestion. A characteristic property of the blood is to keep its specific

NOTE.—Bergtold, W. H. (*Phil. Month. Med. Jour.*, June, 1899), has made a [illegible]

gravity constant. Thus, after drinking large amounts of fluid, the change in specific gravity disappears in from one-half to one hour. Sherrington and Copeman found that after injecting large quantities of salt solution into the veins of rabbits, the specific gravity of the blood returned to the normal in a very short time. It was discovered by Hoch and Schlesinger that a marked change in the specific gravity of the blood as a whole might take place without affecting that of the serum. Therefore it appears that, usually, the principal factors in a change of specific gravity of the blood from the normal are the cellular constituents.

According to Ziegelroth[34], the specific gravity of the blood and of the body as a whole are nearly the same; that of the former being on the average, 1.057 and of the latter, 1.055. The specific gravity of the blood is much more stable than that of the body, as a whole. Copious sweating, he found, had no influence on the specific gravity of the blood.

Of the solid constituents of the blood, in man, the hemoglobin constitutes about 60 per cent. (Hoppe-Seyler). In females it is somewhat less. This being the case, variations in the percentage of hemoglobin must immediately affect the specific gravity of the blood. Hammerschlag maintains that it is therefore possible to determine from the specific gravity, accurately enough for clinical purposes, the hemoglobin percentage for a given sample of blood and has formulated the following table of specific gravities with hemoglobin equivalents.

Specific Gravity.	Hemoglobin, Per cent.	Specific Gravity.	Hemoglobin. Per cent.
1.033–1.035	25–30	1.048–1.050	55–5
1.035–1.038	30–35	1.050–1.053	65–70
1.038–1.040	35–40	1.053–1.055	70–75
1.040–1.045	40–45	1.055–1.057	75–85
1.045–1.048	45–55	1.057–1.060	85–95

According to Hammerschlag, this table holds best for cases of anemia, including chlorosis, tuberculosis and malignant tumors. But in interstitial nephritis, the specific gravity is relatively lower than the hemoglobin. In circulatory disturbances, even when edema is present, the specific gravity is generally normal. In fever, it is relatively lower than the hemoglobin, rising after the fall of the fever. According to Monti[24], a normal specific gravity occurred with a diminution of hemoglobin in chlorosis, mild anemia, heart lesions with anemia and in tuberculosis with evening temperature; a higher specific gravity and normal hemoglobin in developing acute fevers; a high specific gravity and diminished hemoglobin in acute and

chronic diseases, of long duration; a lower specific gravity and normal hemoglobin in acute nephritis with dropsy; a low specific gravity and lower hemoglobin per cent. in leukemia and pernicious anemia; a low specific gravity and a much lower hemoglobin percentage in severe anemias and chlorosis.

Out of the 100 consecutive cases taken for comparison of the three methods (v. Fleischl, Gowers and specific gravity) in our series of 1895, the amount of hemoglobin estimated from the specific gravity and as determined by the Fleischl method differed by less than 5 per cent. in 24 cases: between 5 per cent. and 10 per cent. in 24 cases; between 10 per cent. and 20 per cent. in 26 cases; and more than 20 per cent. in 26 cases. The specific gravity method ran below the Fleischl in but 15 cases and in 5 of these less than 5 per cent. Therefore the difference observed usually showed the readings higher by the specific gravity method than by the Fleischl instrument.

In the same cases the readings of Gowers's apparatus differed by less than 5 per cent from Fleischl's in 36 cases; between 5 per cent. and 10 per cent. in 21 cases; between 10 per cent. and 20 per cent. in 33 cases; and by more than 20 per cent. in 10 cases. As a general rule they were higher than with Fleischl's, but not quite as high as those estimated from the specific gravity. Comparing the percentage obtained from the specific gravity with the Gowers's readings, in 33 cases there was less than 5 per cent. difference; in 31 cases the difference was between 5 per cent. and 10 per cent.; in 26 cases between 10 per cent. and 20 per cent.; and more than 20 per cent. in 10 cases.

From the foregoing comparison, it appears that in approximately one-half of our cases the hemoglobin as determined from the specific gravity corresponded quite well with the hemoglobin as determined by the Fleischl hemometer; that this correspondence was fair between the specific gravity determinations and the Gowers determinations in more than one-half of our cases; that there was a somewhat closer correspondence between the determinations from the specific gravity and the Gowers than there was between the Gowers and Fleischl instruments; that the Fleischl instrument gave relatively lower readings.

The differences in the readings of the three methods may be due: (1) to errors in the specific gravity method; (2) to the inaccuracy of the table giving the hemoglobin equivalent for the specific gravity. (3) to the errors of the v. Fleischl and Gowers instruments.

We are convinced that both v. Fleischl's and Gowers's instruments, particularly the latter, are liable to err to a considerable degree.

Osler says that the error in the Fleischl instrument may not be more than 2 per cent. in blood which is nearly normal, but he cites Neubert and Letzius as having shown that in a much impoverished blood the error may be as high as 20 per cent. In using two Fleischl instruments in comparison in the same cases, we have generally found a difference in reading between the two. In 30 per cent. of these comparisons the difference was as much as 10 per cent. We have also found that in about one-fifth of our cases we disagree in our readings of the same instrument; 40 per cent. of these differences were more than 5 per cent.

We feel some hesitancy in stating that in consecutive tests, made with the same Fleischl instrument, in the same cases, within a few minutes, we have found differences in readings of as much as 10 per cent. This occurred to us after having used the instrument almost daily for nearly a year, and after having used it many hundred times. Such errors can only be explained by acknowledging inaccuracy in technic, but a method so easily liable to such serious errors could hardly be relied upon for more precise results in the hurry of ordinary clinical work.

In the same series of consecutive tests, the specific gravity and the hemoglobin percentage as determined by it, varied to an insignificant degree. The hemoglobin percentages, as determined by Gowers's instrument, in some of the tests, varied as much as 10 per cent. In our experience with the Gowers instrument, we have found it very unsatisfactory. It is often quite impossible to get the tint of the diluted blood to correspond with that of the standard 1 per cent. solution. Even when this is attained, a difference in shade may be produced by looking at the instrument somewhat from the side instead of from straight in front, by holding the paper for reflection farther away from or nearer the instrument, by holding the instrument between the eye and the window, or by moving farther away from the window. In the last case, in a number of instances, the readings obtained by moving 20 feet away from the window became as much as 15 per cent. higher. Therefore it would seem that on a cloudy day readings would be higher than on a bright day. The differences in readings between holding the instrument against a white background and holding it directly between the eye and the light were as much as 10 per cent.[1]

As may be seen from the foregoing comparisons and the observations of others the Fleischl instrument is not entirely reliable; there-

1 See Limbeck, pp. 18, 19, for analogous observations.

fore Hammerschlag's table, which is based upon this instrument, must be inaccurate. Taking all these conditions into consideration, there appears to be quite a constant correspondence between the specific gravity and the percentage of hemoglobin, and we think that, with a table based upon a much larger number of cases and a more reliable estimation of the amount of hemoglobin, this method would be as accurate for clinical purposes as the methods now in vogue, and possibly more so.

Cora Lichty, *Philadelphia Med. Jour.*, 1898, ii., 242-44, gives the following table of specific gravity and hemoglobin equivalents.

Hammerschlag's	Hemoglobin	Method.	Hemoglobin Per cent.
[illegible]	[illegible]	[illegible]	[illegible]

Yarrow and Hitchens[32] have formulated the following table, using standard solutions of hemoglobin for comparison:

Specific	Hemoglobin	Specific	Hemoglobin
[illegible]	[illegible]	[illegible]	[illegible]

They also strongly recommend because of its accuracy Oliver's tintometer for determining the hemoglobin.

In the following table we have recorded a new series of cases. All of these with the exception of 21, which were kindly placed at our disposal by Dr. Albert E. Woehnert, were made at the Erie County Hospital, and we are indebted to Dr. C. R. Orr for a portion of these records.

In the thirty-one records of persons in apparent health, the greatest difference between the hemoglobin by the v. Fleischl instrument and that estimated from the specific gravity was 19 per cent. The discrepancy in this case was probably due to error in the v. Fleischl reading, since the estimated hemoglobin corresponded very closely to the number of red corpuscles.

In eleven cases there was practically no difference between the estimated and determined hemoglobin. In fifteen of the remaining thirty-one cases the difference was less than 10 per cent. In the simple anemias the correspondence between the two was, as a rule, very close. One case, however, showed a difference of 15 per cent., the determined hemoglobin being higher than that indicated by the specific gravity.

The correspondence in the pernicious anemia, as well as in the chlorosis cases, was close. The carcinomas and sarcomas showed no characteristic difference between the estimated and the v. Fleischl hemoglobin. The three stages of syphilis showed a somewhat higher specific gravity than the percentage of hemoglobin (v. Fleischl) would indicate. In the case of pulmonary tuberculosis with afternoon temperature, the hemoglobin percentage (v. Fleischl) was lower than that estimated from the specific gravity. In the nephritis cases, the difference was slight. In three cases of circulatory disturbance, the hemoglobin per cent. was much lower than that indicated by the specific gravity and in four cases there was practically no difference. In the pregnancy records, the hemoglobin was slightly less than would be indicated by the specific gravity. In the cases of epilepsy, the specific gravity estimated hemoglobin was higher than that shown by the hemometer. In appendicitis, the estimated hemoglobin was lower than that determined by the hemometer; in one case slightly higher. In the remaining cases there was no characteristic difference.

TABLE OF RECORDS.

Case number	Specific gravity	Hemoglobin per cent. by Hammerschlag's method	Hemoglobin per cent. by Fleischl's method	Blood Count.		Diagnosis.	Remarks.
				Red	White		
1	1					M H	
2	1						
3	1					H	
4	1						
5	1					M	
6	1						
7	1						
8	1						
9	1						
10	1059	91.67	90				
11	1						
12	1						
13	1						
14	1						
15	1			5,400,000			

TABLE OF RECORDS

												Remarks.
16	.058						M					
							I					
	.058	88.34	75				M					
	.058		74									
		85										
31		85			8,400		F					
24		85	73									
			73									
		85	66									
			84				M					
	.31	25					I					
25		33										
		33										
		75										
		25										
32		66.33	54									
33								Simple anemia..				
34												
35	.050											Improved.
			85									
							M					
	.050		75									
1		11	75				I					
4		11										
		11	70									
43		11										
44												
45												
46												
47		67.6										
48		66.3										
49			50		5750,000			Subphrenic abscess.				
50												
51	.050											
52	.045		35									
53		45			3,450,000							
54			45									
55								Pernicious anemia.				
							M					
57	.046	48										
58			55									
		41	33									
61		41	35									row, strychnine,
					2,520,000							
							I					
64		75										
	.055	75						Chlorosis.				
		95	85				M					
67			93				I	Carcinoma—stomach.				
68								Sarcomatosis.				
		41					M					
								Sarcoma in axilla.				
			75									
		95					I					
	.059		85									
70			64				I					
71			78									
	.045	75					F					
77			45									
78												
							M					
							I					

TABLE OF RECORDS—(*Continued*).

Case number	Specific gravity	Hemoglobin (Haldane)	Hemoglobin (Tallqvist)	Red	White	Sex	Diagnosis.	
81	1.037	85				M		
82	1.037	85	73					
83	1.037	85						
84	1.035	80	77					
85	1.035	75	76					
86	1.037	75						
87	1054	71.6	57					
88	1.033		54					
89	1.032					F	General tuberculosis.	
90	1.036	65	46			M	Pulmonary tuberculosis.	
91	1044	44				F		
92	1056	77.1					Nephritis, chronic interstitial.	
93	1056	77.1	73					
94	1055	75	75					
95	1054		68					
96	1.034					M	Chronic nephritis.	
97	1.040					F		
98	1.040	95				M	Cardiac asthma.	
99	1.040	95					Valvular disease.	
100	1.034							
101	1058					F	Dilatation heart.	
102	1.034						Aortic insufficiency, congestion and pericarditis.	
103	1.044	48		5,300,000	9,375	M	Aortic insufficiency, malaria	
104	1.044	48		2,500,000	1,500		Valvular lesion.	
105	1.040	95				F		
106	1.034	82						
107	1.035	82	75					
108	1.038		77					
109	1.032			409,000	18,750			
110	1.034	92.3				M	Enteric fever.	
111	1.035							
112	1.038		87					
113	1.040	86				F	Epilepsy.	
114		95				M		
115	1058	90						
116	1.038					F		
117	1.037							
118	1.035		75					
119	1056	77.1					Appendicitis.	
120	1052	67.5	86	5,500,000	12,500	M		
121	1.040		62	2,380,000	10,930			
122	1.034					F	Neurasthenia.	
123	1.038					M		
124	1.037	85	85			F		
125	1.038		83			M		
126	1.040		78			F		
127		95				M	2d infection after operation.	Inguinal hernia.
128	1.032					F		
129	1.035	75					Empyema.	
130	1.034		80	5,100,000		F	Chronic suppurative mastitis.	
131	1.038							
132		77.1				M	Septic thrombosis, vein	
133	1.032			4,880,000		F	Myocitis (suppurative), axilla.	
134		95					Chronic diarrhea.	
135	1.035						Addison's disease.	
136	1058		73				Melancholia.	
137	1.038		84				Multiple sclerosis.	
138	1.038			5,487,500	28,229		Lobar pneumonia.	
139	1056	77.1	77	4,337,500	11,714		Hodgkin's disease.	
140	1.035	75				F	Rheumatism (chronic).	
141	1.035	65			10,625	M	Gangrene of feet.	
142	1.037	50		3,900,000	14,800	F	Microcephalus.	

A fair idea of the relation of the specific gravity to the percentage of hemoglobin in the blood is given in Menicanti's conclusions and in those of Dieaballa.

Menicanti[22] says: "In health there is a certain constant relation between the specific gravity of the blood and its percentage of hemoglobin, with very small and varying individual differences. The same holds good in chlorosis, in ordinary anemias and other diseases. In pregnancy and often in heart disease, the relation varies, so that the same percentage of hemoglobin denotes a lower specific gravity."

Dieaballa[7] concludes:

1. The specific gravity of the blood depends, in the first place, upon its hemoglobin contents, but with the same quantity of hemoglobin differences as high as 13.5 per mille specific gravity may occur.

2. 10 per cent. hemoglobin (according to v. Fleischl) denote a specific gravity of 4.46 (according to Hammerschlag.)

3. With the same percentage of hemoglobin, both under physiological and pathological conditions, in women the specific gravity is from 2 to 2.5 lower than in men.

4. The specific gravity varies within wider limits in blood that is rich in hemoglobin, than in blood that is poor in hemoglobin.

5. In nephritis, owing to the hydremia of the plasma, the specific gravity is from 4 to 5 per mille lower than in blood of the same hemoglobin percentage and number of corpuscles in secondary anemia.

6. In leukemia the specific gravity is higher than would be excepted from the percentage of hemoglobin. This difference appears to be in proportion to the greater number of leucocytes.

7. In chlorosis, the specific gravity is about 2.5 per mille higher than in secondary anemia. This difference rapidly disappears during blood regeneration.

8. In those forms of pernicious anemia, where the number of red blood corpuscles is appreciably diminished in relation to the hemaglobin, the specific gravity is about 2 per mille lower than in secondary anemia. During blood regeneration this disappears.

9. The number of red corpuscles in the blood may have a positive influence upon the specific gravity, independently of the hemoglobin, since with the same percentage of hemoglobin differences in specific gravity of 4 to 5 per mille may be produced from this cause.

Our conclusions may be briefly summarised as follows:

1. In most cases the specific gravity and the percentage of hemaglobin of the blood present such a close relation to one another that the latter may be predicted from the former with sufficient accuracy for clinical purposes.

2. Both v. Fleischl's and Gowers's instruments are liable to an error of 10 per cent. or more.

3. There is liable to be very slight if any error in the determination of the specific gravity by Hammerschlag's method.

BIBLIOGRAPHY.

1. *Arronet*: Malv. Jahresbericht für Thierchemie, 1882.

2. *Becquerel and Rodier*: Recherches sur la Composition de Sang. Deutsch von Eisenmann, Erlangen, 1845.

—— —— Traité de Chimie Pathologique. Paris. 1854, p. [illegible].

3. *Castellano, P. F.*: Sulla Densita del Sangue, Gazz. d. Osp Milan, 1892, xiii., 718-790.

4. *Copeman, I. M.*: Report on the Specific Gravity of the Blood in Disease. British Med. Journ., London, 1892, i., 961-963.

5. *Dastre, A.*: Methode Nouvelle pour la Determination de la Densite du Sang. Arch. de Physiol., 1893, p. 791; also extract Centralb. für die Med. Wiss., Vol. xxxii., p. 461.

6. *Dekio, K.*: Zur Kritik des Fleischlchen Hämometer. Verhandl. d. Cong. f. Innere Med., Wiesb., 1892, xi., ii., 135-143.

7. *De Lote, J.*: Ueber d. Dichte d. Blutes unter Pathologischen Verhältnissen. Ztschr. f. Heilk., Berlin, 1890, xi, 175-186.

8. *Dieaballa*: Deutsches Archiv. f. Klin. Med., 1890, Bd. 57.

9. *Erkman*: Blutuntersuchungen in den Tropen, Virchow's Archiv., Bd. [illegible], 1892, p. [illegible].

10. *Fano, G.*: Di una nuova funzionei de corpuscoli rossi de Sangue Sperimentale. Firluze, 1882, i, 256, 370

11. *Glogner*: Specifische Gewicht des Blutes in den Tropen, Virchow's Archiv., Vol. 126, p. 109.

12. *Hammerschlag, A.*: Ueber das Verhalten des Specifischen Gewichts des Blutes in Krankheiten. Centralblt. für Klin Med., Leipzig, 1891, xii., 825-837.

13. ——— Eine Neue Methode zur bestimmung des Specifischen Gewichts des Blutes. Ztschr. f. klin. Med., Berlin, 1892, xx., 141-150.

14. *Koch A. und Stenberger, H.*: Hamatologische Studien. Beitr. z. Kinderk. und Prot. Kinder Rr. Qust in Wien, 1892, n. F. ii, 1-62.

15. *Hoppe-Seyler*: Beiträge zur Kenntniss der Eigenschaften der Blutfarbstoffe. Ztschr. f. Physiol. Chem., Strassburg, 1881, Vol. [illegible].

16. *Jones, F.*: On Variations in the Specific Gravity of the Blood in Health. Journ. of Physiol., Cambridge, 1887.

17. ——— Further Observations on the Specific Gravity of the Blood in Health. Journ. of Physiol., Cambridge, 1891, xii., 299-346, 4 pl.

18. *Landois and Stirling*: Textbook of Human Physiology. Translated from the German edition, London, 1891, 4th edition.

19. *Limbeck v. R. R.*: Grundriss Einer klinischen Pathologie des Blutes. Jena, 1892. Verlag von Gustav Fischer.

20. *Lyonnet B.*: De la Densiti du Sang, la Determination Clinique ses Veriations Physiologiques et Pathologiques. Paris. 1892.

21. *Lichty, Cora*: Philadelphia Med. Jour., 1898, ii., 242-44.

22. *Menicanti*: Ueber das Specifische Gewicht des Blutes und dessen Beziehung zum Hemoglobingehalt. Deutsches Arch. f. klin. Med., Leipzig, 1882, i., 407-422.

23. ——— Ueber das Specifische Gewicht des Blutes und dessen Beziehung zum Hemoglobingehalt. Arb. a. d. med. Klin. Inst. d. Univer. zu München, Leipzig, 1893, iii., 490-503.

24. *Monti*: Veränderungen der Blutdichte bei Kindern. Abst. Schmidt's Jahr., Vol. 250, p. 189.

25. *Peiper*: Das Specifische Gewicht. des Menschlichen Blutes. Centrabl. f. Klin. Med., 1891, No. 12.

26. *Roy, C. S.*: Note on a Method of Measuring the Specific Gravity of the Blood for Clinical Use. Edinburgh Clin. and Path. Journ., 1883-4, i., 561-563.

27. *Schmaltz, R.*: Das Verhalten des Specifischen Gewichts des Blutes bei Kranken. Deutsche Med. Wochenschr., Leipzig, 1891, vxii., 555-559.

32. *Warren and Hutchens:* Clinical Estimation of the Quantity of Hemoglobin in Human

135 ALLEN STREET.

Extracted from The American Journal of the Medical Sciences, June, 1900.

A CRITICAL SUMMARY OF RECENT LITERATURE

ON

PLASMA-CELLS AND MAST-CELLS.

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The two classes of cells which are the subject of this paper have been studied with much care during the past few years and by numerous observers. It is hoped that a summary of the facts they have recorded will be considered timely. The significance and functions of these cells are still problematical for the most part. However, we may be quite certain that they have important work to do, and we may confidently expect to have much light upon them in the near future.

Plasma-cells.

In 1891 Unna[32] described the elements which he called "plasma-cells," believing them to be the same as certain granular connective tissue cells, to which Waldeyer had previously given this name. Later studies indicated that the two were not identical, and Waldeyer[40] advised the restriction of the term "plasma-cell" to that of Unna, finding that the structures named by himself (Waldeyer) corresponded, at least in large part, with the "mast-cells" of Ehrlich, which will be considered below.

Morphology. The plasma-cells of Unna are peculiar in having *protoplasm* that is stained by basic aniline dyes. With Unna's polychrome methylene-blue (described hereafter) the plasma-cells are stained blue-violet, while their nuclei are blue, according to most writers. The outer part of the protoplasm stains more deeply than the inner part, leaving a pale zone around the nucleus. Unna first described the plasma-cell as containing numerous stained granules in its protoplasm. Such granules have not been seen by most other observers. It is not uncommon, however, to find plasma-cells whose protoplasm is not homogeneous, but which contain small clumps and particles that stain somewhat unequally and irregularly.[19] The nucleus is round or oval, and is usually placed eccentrically. Five to eight deeply stained masses of chromatin are arranged chiefly about the border of the nucleus. Some observers have described a nucleolus. The appearance of the nucleus depends much on the manner of staining and decolorizing. Two or more nuclei are occa-

sionally present. In size the plasma-cells vary from being of the dimensions of leucocytes to much larger—average diameter 6–7 $\mu \times 8$–10 μ.[17] In shape they are round, oval, roughly cubical or elongated, according to whether or not they are limited by connective tissue fibres or the pressure of other cells. Krompecher's[19] illustrations of the different forms of plasma-cells are excellent. The above description of the plasma-cell differs somewhat from Unna's and follows that of v. Marschalkó[23] and others who have written on this subject more recently. Undoubted indirect cell-division seems to have been observed only by Councilman[9] and Mallory,[22] but amitotic division has often been mentioned.[19] Degenerative changes in plasma-cells have been described by a number of observers.[17]

Cells whose protoplasm may be stained, but which differ from plasma-cells more or less in other respects—"pseudoplasma-cells"—have been described.[13,19] Their significance is at present not clear.

Occurrence. Although they were supposed at first to belong only to pathological conditions, plasma cells have been reported as occurring in lymph-nodes, in the lymphoid tissues of the spleen, and in the bone-marrow, both in man and the lower animals,[16] in ligaments,[28] in the framework of mucous glands of the tongue,[17] and especially in large numbers in the mucosa of the stomach and intestine in man.[9] Information as to their frequency and distribution in normal tissues is not very full. On the other hand, much has been written concerning their occurrence in diseased conditions, and a mere enumeration of these conditions would require several pages. A description of the pathological histology of any lesion can hardly be considered complete now which does not take account of the plasma-cells, if they are present.

Unna's first accounts of plasma-cells were based on sections of lupus. He early directed attention to tumor-like collections of these cells; such a collection he called a "plasmoma." It has appeared since that plasma-cells are abundant in the lesions of tuberculosis, wherever situated, in those of syphilis, leprosy, actinomycosis, and rhinoscleroma[39]—*i. e.*, the so-called "infectious granulomata." They are also frequently seen in large numbers in the stroma of carcinoma. In the main they are most characteristic of rather chronic processes, where they constitute an important part of what is usually termed round-cell infiltration. In acute inflammatory conditions, and especially in acute suppuration, they appear in smaller numbers, or are absent.[17] But v. Marschalkó[23] and Joannovics[17] were able to demonstrate plasma-cells in acute inflammatory lesions artificially produced in animals. Justi[18] found them in recent granulating wounds in man and the dog. Mallory[22] found them in the lesions of typhoid fever in the intestines, spleen, lymph-nodes, etc. According to Councilman,[9] plasma-cells constitute the bulk of the cell-infiltration in acute interstitial nephritis.

The study of the plasma-cells in the diseases of the skin has been carried on by Unna with great energy, and for the results of his work his *Histopathology of the Skin*[39] may be consulted.

Origin, Significance, and Function. Concerning the origin of the plasma-cells, Unna[32] maintained that they were derived from connective tissue cells, stating that he was able to detect all the necessary transitional forms between the two. Most observers, however, believe that the plasma-cells are derived from the lymphocytes, chiefly the small mononuclear variety.[9 16 17 18 19 23 28] Possibly a few originate from polynuclear leucocytes.[19 17] Furthermore, it is claimed that the plasma-cells may become changed to connective tissue cells, and thus aid in the formation of fibrous tissue.[23 19 28 12] This latter hypothesis would make the production of connective-tissue cells from lymphocytes possible under certain circumstances, the plasma-cells being an intermediate form. It would modify existing ideas very considerably, and convincing proofs will be demanded before it can be accepted. It rests in part upon the same evidence as the theory of Unna above mentioned, namely, the existence of what appear to be transitional forms between plasma-cells and connective tissue cells. For a full discussion of this question, see the papers of Krompecher[19] and v. Marschalkó.[23]

Joannovics[17] takes a middle ground, believing that part of the plasma-cells come from lymphocytes and part from connective-tissue cells; the latter may become connective tissue cells again, while the former undergo degenerations of various sorts. It is not claimed by anyone, I believe, that all or most plasma-cells are destined to become connective tissue cells. The function or significance of these bodies is, therefore, at present uncertain. It is to be noted that they are not distinctive of any particular disease or class of diseases. Their absence from acute suppuration is remarkable. According to Councilman[9] and Mallory[22] they have amœboid properties; they may occur inside the bloodvessels, and may emigrate from the vessels by amœboid movement. Howard[15] states that he has demonstrated that plasma-cells may become transformed into typical eosinophiles, basing his opinion chiefly upon his studies of a case of trichinosis.

If the derivation of plasma-cells from lymphocytes be accepted, we shall have another clue to the origin of the cells in inflammatory exudates. Unna regarded the plasma-cells as being the expression of a degenerative change, whereas v. Marschalkó[23] considered them as evidence of progressive activity in the protection of the organism. Justi[18] thought it likely that they serve to eliminate some unknown substance by way of the lymphatics. Joannovics[17] suggested that their staining properties may be due to their having taken up chromatin from other and degenerated cells.

Mast-cells.

The term "mast-cells" was applied by Ehrlich[10] to large cells occurring in connective tissues, the protoplasm of which contained good-sized granules with marked affinity for basic aniline dyes (basophile granules, γ granules of Ehrlich). The name was considered appropriate because these cells occurred abundantly in conditions of congestion and chronic inflammation, and under other circumstances where he supposed nutrition to be heightened. It has already been stated that the plasma-cell of Waldeyer corresponds in the main with the mast-cell of Ehrlich. In size the mast-cells vary from that of a large leucocyte to many times those dimensions. They may be rounded, or they may present extremely irregular figures, which are determined apparently by the cavities of the tissue which they occupy. Forms shaped like an hour-glass or with several long, sinuous processes are common; they remind the observer of the outlines of a moving amœba. Their occasional occurrence in the epithelial layers of the skin can hardly be explained except by supposing that they have the power of amœboid movement.[2 3] There is a relatively small, round nucleus, somewhere near the middle of the cell, which is usually in part, and sometimes wholly, obscured by the granules. Karyokinesis seems not to have been observed.[2 3] The granules are the most striking characteristic, as they are usually good-sized, stain rapidly and intensely with the basic aniline dyes, and are decolorized with difficulty. They may be stained by Gram's method, and when they are large, as they are usually quite uniform in size, they may easily be mistaken for micrococci. The granules will sometimes retain the dye when the ordinary methods for staining the tubercle bacillus are used. Furthermore, the granules exhibit with stains the phenomenon of metachromism—*i. e.*, of presenting a shade of color more or less different from that which the dye imparts to the nuclei of the mast-cells and of the other elements of the tissue. The metachromism is most marked with thionin and toluidin-blue. These dyes stain the nuclei of cells of all sorts of blue, while the granules of the mast-cells are violet to red. The metachromism is reported to be very marked with kresyl-violet R.[11] The granules of the mast-cells are said by some to have a certain amount of affinity for acid dyes.[4 5] Granular mast-cells are sometimes seen which are surrounded by a stained halo of the same color as the granules;[11 14 39] also free granules in the vicinity of mast-cells, and mast cells with protoplasm that stains as well as the granules.

The nature of these granules is undetermined. Hoyer[16] tentatively revived an earlier theory of Raudnitz, to the effect that the granules consisted of mucin. After an exhaustive study of the staining properties of mucin, he found that the metachromism shown by mucin and by the granules of mast-cells after thionin staining was very similar.

Unna[36 38] appears to regard this view with favor; it has recently been advocated by Harris[12]. It is to be noted that mucin is extremely capricious in exhibiting metachromism, and that the products of amyloid and other degenerations also show varying degrees of metachromism.

Basophilic granules may occur in blood-cells which are not mast-cells, as in the lymphocytes of normal blood after prolonged staining,[29] and the perinuclear basophilic granules described by Neusser.[6] According to Litten,[20] basophilic granules occur sometimes in the red blood-corpuscles in cases of anæmia; they differ from the granules of the mast-cells in not being metachromatic.

Occurrence. Unlike plasma-cells, the mast-cells are very widely distributed in normal tissues. They have been observed in many different vertebrates, from the frog to man. According to Ehrlich,[10] the mast-cells are likely to be found just under epithelial surfaces, and immediately about the bloodvessels. They appear in the blood of the frog, and are abundant in the frog's tongue. In those animals in which mast-cells occur at all they are likely to be especially numerous in the tongue, as in the case of the bat; in the striped muscles generally they are not numerous, nor are they particularly so in the heart.[10] The rabbit, hare, and guinea-pig are remarkable for the small number of mast-cells in their tissues.[10]

In man the mast cells occur at all periods of life,[26] even in the newborn.[2] While they are comparatively uncommon in early infancy, they are always more or less numerous in the adult. In normal blood they are present in very small numbers and are probably derived from bone-marrow.[11] They constitute part of the cells of bone-marrow. They occur between the fibres of uterine muscle[27] and of the muscle of the intestine. They are usually to be found in the mucous membranes, where they are often numerous. They always occur in the skin, frequently in abundance. They are seen in the tissues of the normal resting mammary gland,[8] also in the active mamma, and, according to Unger,[30] are important in the transportation of fat in the mamma. They occur in the testicle, except in infants under about four months of age.[24] In pathological conditions they exhibit a certain amount of parallelism with plasma-cells in the manner of their occurrence. They are not characteristic of acute inflammation, especially not of acute suppuration.[26] However, Neisser[11] saw a case of gonorrhœa in which the pus consisted only of mast-cells. Also, according to Coen,[7] they appear in the skin in increased numbers, as the result of the application of strong solutions of iodine. They are often numerous about the lesions of chronic infectious processes, such as tuberculosis, but not necessarily; and they are by no means as characteristic of them as are plasma-cells. They are frequently abundant in the stroma of carcinoma, especially of the breast and skin where the fibrous tissue is plentiful. They are

generally numerous in uterine fibromyomata[7] and in neurofibromata,[39] and in processes where cicatricial fibrous tissue is forming, as cicatrizing gastric ulcer. They are usually abundant in the mucous membranes of chronic catarrhs, and in the lung of cyanotic induration.[10] According to Ehrlich, stagnation of the lymph-stream favors their accumulation, as in elephantiasis. Cells with basophilic granules occur in normal blood in very small numbers, and are usually supposed to be the same as mast-cells; in leukæmic blood they are not rare, but their significance in leukæmia is not known.[6 29] They are constantly and greatly increased in myelogenic leukæmia.[11]

Origin, Significance, and Functions. It has been suggested that mast-cells are wandering cells that have taken up disintegrating masses of chromatin; that they have some relation with pigmentation, especially in the skin (Phillipson, cited by Baumer);[5] that they perform an eliminative function in inflammation (Metschnikoff, cited by Baumer);[5] also, that they may be formed by a metamorphosis of unstriped muscle-cells.[27] It is noticeable that the mast-cells seldom collect together in large masses, as plasma-cells often do. According to Unna,[9] urticaria pigmentosa forms a striking exception to the usual condition, as its lesions contain accumulations of mast-cells. This observation has been confirmed by others, including Baumer,[5] who secured a somewhat similar condition by the repeated action of urtica urens on his own skin. Baumer believed that the mast-cells originate from the connective tissue cells in the adventitia of the bloodvessels; these connective tissue cells showed numerous karyokinetic figures.

Concerning the significance and functions of mast-cells we are almost wholly in the dark. The staining properties of the granules are usually supposed to depend on peculiarities in their chemical composition. Ehrlich and Lazarus[6] regard them as a variety of granular leucocytes; according to Ehrlich's theory, the granules are products of the specific activities of these cells, resembling a secretion; each kind of leucocyte contains only one kind of granule; it is probable that the granules of the wandering cells are destined to be given off into the surrounding structures. From a recent article of Arnold,[1] it appears that he doubts whether all the cell granules are secretory in nature.

Technique.

The fixation of the tissues may be accomplished in alcohol,[33] corrosive sublimate,[23] or Zenker's fluid.[9] Müller's fluid is apparently not applicable. The plasma cells are shown well after fixation in 4 per cent. formaldehyde, or in Müller-formol, but the staining of the granules of the mast-cells is modified as mentioned below.

Unna[37] explains the metachromism shown by the mast-cell granules and other bodies by supposing that solutions of the aniline dyes contain

small quantities of other dyes as impurities. His alkaline methylene-blue, or "polychrome methylene-blue," is an extreme example, and he states that it contains methylene-violet and methylene-red in addition to methylene-blue.

Unna[33] has given several formulas for making polychrome methylene-blue, of which the following will be found satisfactory; it must stand several weeks to months before using:

Methylene-blue	1
Potassium carbonate	1
Distilled water	100

Sodium or ammonium carbonate may be used instead of potassium carbonate. To obtain the best results the specimens should be over-stained, the excess of color being removed with some decolorizing agent.

Stain in the methylene-blue solution, which may or may not be diluted, fifteen minutes or longer.

Rinse in distilled water.

Decolorize in water to which a few drops of "glycerin-ether" have been added, for a quarter of a minute, or for several minutes, as required, to show beginning differentiation of the structure.

Complete the decolorization with alcohol.

Clear in oil of bergamot or xylol.

One-half to one per cent. acetic acid solution appears to serve as well as glycerin-ether, and alcohol alone nearly as well.

By the above staining process the plasma-cells are stained a blue-violet, their nuclei and other nuclei blue, the granules of mast-cells red. Epithelial cells take the blue stain, sometimes intensely, especially the horny layers of the epidermis; giant-cells are frequently stained as well; also, the products of various degenerations, as amyloid and mucoid, which may show varying degrees of metachromism.[17] The development of a red color in the granules of the mast-cells is favored by extreme decolorization.

Nearly identical results, including the red color of the granules of the mast-cells, may be secured by employing watery solutions of thionin[16] or toluidin-blue, decolorizing with alcohol, or an acid followed by alcohol.[32] The plasma-cells often stain fairly well with ordinary methylene-blue solutions, and sometimes even with the other basic dyes. With basic dyes other than thionin, toluidin-blue, and polychrome methylene-blue, the granules of the mast-cells are well stained, but they are less easily distinguished, because the metachromism is much less pronounced. The mast-cell granules sometimes stain well with hæmatoxylin, but it is not clear under what conditions this occurs.

The writer has found that after fixation with formaldehyde or Müller-formol the mast-cell granules, after staining with polychrome methylene-blue, thionin or toluidin-blue, are colored *blue* or *violet* and *not red*, as occurs after alcohol or sublimate fixation, using two pieces of the same

tissue, one fixed with alcohol, the other with formaldehyde, stained in the same solution; the granules of the mast-cells will be much redder in the specimen fixed by alcohol. This difference is most obvious after prolonged fixation and with frozen sections. It is more or less modified by paraffin or celloidin embedding, although the differences in color usually remain quite distinct after embedding. It appears equally well after decolorization with alcohol, acetic acid, and glycerin-ether, provided the decolorization is prolonged sufficiently. This work will be described in detail elsewhere.

In staining cover-glass preparations of blood, it is to be remembered that the granules of the mast-cells are not stained by the Ehrlich tricolor[11] mixture, although they may be stained by the simple basic dyes, such as methylene-blue.

References.

[illegible]

5. Birch-Hirschfeld. Allg. path. Anat., 1896, p. 156.
6. Cabot. Clinical Examination of the Blood, 1897.
7. Coen. Ziegler's Beitr., 1887, Bd. ii. p. 31.
8. —— Ziegler's Beitr., 1887, Bd. ii. p. 85.
9. Councilman. Journ. Exp. Med., 1898, vol. iii. p. 393.
10. Ehrlich. Farbenanalytische Untersuchungen, etc., des Blutes, 1891.
11. Ehrlich and Lazarus. Normale und path. Hist. des Blutes, Specielle Path., etc., Nothnagel, 1898.
12. Harris. Philada. Med. Journ., 1900, vol. v. p. 757.
[illegible]
14. Hoyer. Arch. für mikroskop. Anat., 1890, Bd. xxvi. p. 310.
15. Howard. Philada. Med. Journ., 1899, vol. iv. p. 1085.
16. Jadassohn. Berl. klin. Wochenschr., 1893, Bd. xxx. p. 222.
17. Joannovics. Zeitschr. für Heilkunde, 1899, Bd. xx. p. 159.
18. Justi. Virchow's Arch. für path. Anat., etc., 1898, Bd. cl. p. 197.
19. Krompecher. Ziegler's Beitr. zur path. Anat., etc., 1898, Bd. xxiv. p. 163.
20. Litten. Deutsch. med. Wochenschr., Jahrg. 25, 1899.
21. Mallory. Journ. Exp. Med., 1897, vol. ii. p. 529.
22. —— Journ. Exp. Med., 1898, vol. iii. p. 611.
23. v. Marschalkó. Centralbl. für allg. Path., etc. 1899, Bd. x. p. 851.
24. Munchheimer. Fortschritte der Medicin., 1895, Bd. xiii. p. 104.
25. Neisser. Arch. für Dermat. und Syph., 1895, Bd. xxxi. p. 387. (Personal in character.)
[illegible]
30. Unger. Virchow's Arch. für path. Anat., etc., 1898, Bd. cli. p. 159.
31. Unna and Vanderspek. Monatshefte für prakt. Dermatologie, 1891, Bd. xiii. p. 304.
[illegible]
Berl. klin. Wochenschr., 1893, Bd. xxx. p. 222.
Monatshefte für prakt. Dermatologie, 1894, Bd. xviii. p. 57.
Monatshefte für prakt. Dermatologie, 1894, Bd. xix. pp. 225, 397, etc.
[illegible]
the original.
41. Westphal. Ueber Mastzellen, Inaug. Diss., 1890. Included in 10.

The Morbid Anatomy and Etiology

of

FAT-NECROSIS

by

HERBERT U. WILLIAMS, M. D.

Buffalo, N. Y., 1900.

SYNOPSIS.

ILLUSTRATIONS.

THE PATHOLOGY AND ETIOLOGY OF FAT-NECROSIS.

THIS report is founded chiefly upon papers which have already appeared in journals, and which will be referred to in the text. It is hoped that it will be useful to those who are interested in the subject to have these observations presented together and in a connected form. Some new facts and accounts of further experiments have also been added. No attempt has been made to review the whole of the very voluminous literature of fat-necrosis. In the papers of Fitz,[7] Körte,[20] Nimier,[24] Katz and Winkler,[19] and Warthin,[36] the bibliography has been given in detail.

In beginning, three cases of disseminated or multiple fat-necrosis in man will be considered. Their clinical histories and pathological anatomy are in most respects characteristic. The writer is indebted for the clinical histories to Dr. Charles G. Stockton. These cases have been reported already by Dr. Stockton and the writer.[33, 34]

THREE CASES OF MULTIPLE FAT-NECROSIS.

CASE I.—"E. H., a male Swede, came to America when eight years old. His parents lived beyond seventy. His early life was spent on a farm; his latter life in a large and lucrative business. He was a stout man of the blonde type, nervous and always active. He had suffered from otitis media, the loss of the right ear-drum, and had for two years experienced the sensation of giddiness, but no tinnitus.

"He tired easily, was flatulent, and slightly constipated. The pulse was frequent and poorly sustained; the cardiac

impulse was obscure and felt to the left of the nipple-line. In twenty-four hours he voided 1428 c.cm. of urine, acid in reaction, containing 15.7 grm. of urea, no albumin, no sugar, and negative microscopically.

"Under the influence of massage, baths, and a regular diet continued one year, his general condition improved, and he was persuaded to take a European trip for rest. The change was enjoyed, and he reported steady improvement, although he apparently displayed as a tourist his characteristic activity. On the eve of his return he visited the canal at Manchester, and reached Liverpool chilled and exhausted. He ate heartily at dinner, but soon after was seized with vomiting, retention of urine, abdominal tenderness and pain. Dr. W. S. Crawford, of Liverpool, catheterized him, and during the night administered seven-eighths grain of morphine hypodermatically. The temperature was 100°, and the circulation feeble. The vomiting continued, with moderate pain and great thirst. The doctor writes me that he believed that the patient suffered from subacute peritonitis. Feeling slightly relieved, the home voyage was undertaken three days after the onset of the attack. The patient grew steadily worse, vomited daily, retained absolutely no nourishment, and required morphine for the relief of pain. The ship's surgeon, Dr. S. R. Radmore, of the 'Teutonic,' felt himself fortunate in turning over the patient alive to the late Dr. Goldthwaite, of New York. The late Dr. Loomis saw the patient the night of his arrival; I saw him the next morning. The striking features of the case at the time were those of prostration, suggesting shock and toxæmia. A possible appendicitis was considered by Dr. Loomis, but he was not prepared to make a positive diagnosis, nor to advise operation. I did not see the patient again for three days, when I returned to escort him to Buffalo. He was then in coma, and died six hours after reaching home.

"There were present during the last days general abdominal tenderness, moderate pain and tympany, no induration, the temperature never above 100°, and the pulse frequent and very feeble. Koumyss, whiskey, and water were retained in

fair quantity. The alvine evacuations appeared natural. Dr. Goldthwaite reported the urine free from albumin and sugar, although Dr. Radmore had found albumin present during the voyage. Catheterization was required before the use of anodyne. The diagnosis was not made. The general expression of the case was not unlike that of uræmia, but there was more abdominal tenderness and pain than seemed consistent with such a condition alone."

Report of Autopsy: October 18, 1894, 4 P. M. E. H., Swede, aged fifty-nine years; dead thirteen hours. Rigor mortis firm in legs, not so well marked in arms; body appears well nourished; post-mortem lividity present over the back; subcutaneous fat 18 mm. thick over the thorax, 30 mm. thick over the abdomen; muscles of thoracic and abdominal walls scantily developed; costal cartilages calcified. Left lung free throughout; right lung adherent at apex; both lungs crepitated well and appeared normal; moderate congestion and œdema posteriorly. No unusual amount of pericardial fluid; valves of heart normal; muscle of left ventricle somewhat thinned, soft; cavity of left ventricle slightly dilated; aortic intima fatty. Spleen small, dark, and friable. Liver rather small, slightly fatty. Gall-bladder small, walls thickened, containing numerous dark gall-stones the size of buckshot. Mucous membrane of stomach, small and large intestines appeared normal; that of the large intestine showed white dots the size of a pin's head, scarcely noticeable; appendix 30 mm. in length, size of chicken-quill in diameter; except for its small size, normal. Kidneys imbedded in abundant fat, the capsule of each stripped off easily; parenchyma pale, cortex narrow; two small cysts on the surface of the right kidney. Urinary bladder contained about four ounces of urine; mucous membrane normal.

Most interesting was the condition of the fatty tissues of the abdominal cavity. The subperitoneal fat and that of the mesentery, mesocolon, and omentum was abundant. Imbedded in the fat of the omentum, mesentery, and mesocolon were great numbers of round, hard, white masses of variable size,

mostly smaller than buckshot, white and opaque on section. In the mesocolon they were extremely abundant, often soft, and becoming confluent, making a pultaceous mass. The same condition prevailed in an extreme degree in the retroperitoneal fat behind the colon, stomach and spleen.

Removal of the spleen revealed a large cavity, extending downward between the left kidney, the colon and the stomach, containing a soft, cheesy substance resembling pus, with masses nearly as large as a pea, of firmer consistence. The fatty tissues bounding this cavity were grayish and necrotic in appearance. In this cavity lay the pancreas, surrounded by the same kind of material, some of which also was adherent to it. The fat about the kidney was sharply separated from the other retroperitoneal fat, and was unaltered, as was the fat of the anterior abdominal and thoracic walls.

The pancreas was eighteen centimetres in length, and to the unaided eye the parenchyma appeared normal; the duct of Wirsung was normally situated and was patulous. Microscopic examination showed its wall to be denuded of epithelium, except in the deeper folds, in which the epithelium refused to stain; the other tissues of the duct also stained badly; no cell-infiltration into it or around it was observed. On microscopic examination of the parenchyma of the pancreas, the epithelium was found to take nuclear stains badly or not at all, in sections made at a number of points between the head and tail. The epithelium of the ducts behaved in a similar manner. The amount of interstitial connective tissue was not unusual. The condition of the parenchyma corresponded closely with the auto-digestion of the pancreas described by Chiari. Over small areas it was moderately infiltrated with fat. A region of this character near the tail showed a small spot of fat-necrosis, having the characters to be described later. The capsule for the most part was not thickened, and exhibited no cell-infiltration; near the tail it was slightly thickened, and showed a small hæmorrhage; it contained numerous round, oval and spindle-shaped nuclei. No bacteria could be demonstrated in this pancreas, except in

a single section from near the head, in which the duct of Wirsung contained numerous short, plump bacilli with rounded ends.

CASE II.—"Eleven days later, October 29th, 1894, there appeared in the ward at the Buffalo General Hospital a Swede, E. A., male, aged thirty-nine years, a laborer. For three weeks preceding he had been under the care of Dr. W. H. Slacer, who reported that the man had been taken ill while at work some weeks prior with abdominal pain and nausea. At the time of examination, October 9th, the pulse was weak, counted 90, and the temperature marked 101°. The patient was emaciated, anæmic, slightly icteric, the tongue heavily coated, constipated, the fæces light yellow and offensive. The urine was said to be natural in color and quantity, specific gravity 1024, with absence of sugar, albumin, and sediment.

"The stomach was distended with gas; there was great tenderness over the stomach and to the right of the umbilicus. There were anorexia, great thirst, and occasional vomiting. Dr. Slacer attended the man for chills and fever one year before, from which he had made a complete recovery.

"When received into the hospital the patient was greatly prostrated, and complained of sharp, lancinating abdominal pain, especially in the region of the umbilicus. There was general abdominal tenderness, slight tympany, but no perceptible induration.

"As to his general condition, the skin was muddy and slightly icteric, the tongue was heavily coated, the pulse varied from 80 to 90 and was very feeble, the temperature was 99°. Time did not permit of a thorough examination of the urine, but it was found free from albumin and sugar. The man was unable to retain nourishment. He was given nutrient enemata, besides digitalis and strychnine hypodermatically. Morphine was repeatedly required for the relief of pain. This case resembled so closely the one previously described that I was much disappointed to find on the following day that the patient was dead, without a diagnosis having been made."

Report of Autopsy: October 31, 1894, 4 P. M. E. A., aged

thirty-nine years; dead fourteen hours. Rigor mortis firm; well formed, but slim; post mortem discoloration well marked in buttocks, back, backs of arms and neck; a number of dark spots in skin of anterior surface of right thigh, right upper arm and left hip. Subcutaneous fat over thorax 6 mm. thick, over abdomen 18 mm. thick; subperitoneal fat well developed. Both lungs free from adhesions; pleural cavity empty; lungs small, crepitated well, and were normal; right weighed 377 grams, left 261 grams. Pericardial cavity free from fluid; heart of normal size, weight 246 grams; usual amount of subpericardial fat; right ventricle contained large yellow clot, valves normal, muscle of ventricle of usual thickness and healthy; aorta moderately atheromatous. Spleen weighed 203 grams, of normal appearance. Liver appeared fatty; centres of lobules congested. Gall-bladder dilated, encroaching on anterior surface of liver, which was notched correspondingly; its shape was unusual, being constricted about the middle, and the cystic duct compressed by a fibrous band; bile passed freely into the duodenum on pressure, however. Mucous membrane of stomach and duodenum normal; that of jejunum congested and œdematous, covered with mucus; ileum less so, except the lower portion, which was congested. About one metre above the ileo-cæcal valve, the ileum was bound down by a recent-appearing adhesion, not very firm. Breaking of this adhesion disclosed behind it a cavity as large as a walnut, containing fluid resembling pus and small, harder masses. Appendix vermiformis 8.8 cm. in length, of which 7.5 cm. were free; tissue about the cæcum normal; mucous membrane of the colon near the splenic flexure necrotic and easily torn; colon beyond this point congested; contents yellowish, semi-fluid. Kidneys weighed 159 grams each; capsules stripped off with ease, appeared a little swollen; cortices very pale, medullæ dark; suprarenal bodies normal in size and appearance.

The peritoneum showed no congestion or exudate; beneath it the fatty tissues of the abdominal wall were sparingly dotted with small, hard, white nodules; the same were present in larger numbers in the mesentery; they were seen about the

cæcum. Removal of the spleen disclosed a mass of necrotic tissue, soft, grayish, containing fluid resembling pus, small, hard, white masses, and others easily crushed between the fingers. This condition extended through the retroperitoneal fat-tissue to the splenic flexure of the colon. The necrotic portion of the colon already mentioned was in contact with black necrotic tissue behind it.

The fatty tissues about the pancreas were included in the same necrotic area. The outer surface of the pancreas was grayish-black, and dotted with the same white masses. The pancreas was 26 centimetres in length; weight, 172 grams. The head was large, quadrilateral, margin angular, sharply separated from the rest of the organ, which was united with the head by a constricted portion, about the size of a lead-pencil. Section of the pancreas showed it to be hard; fibrous tissue present in abnormal amount; parenchyma unusually reddened. The duct of Wirsung was patulous; microscopic examination showed its epithelium, for the most part well stained, but some smaller ducts were filled with desquamated epithelium and small round cells. In the constricted part above mentioned, the duct contained a hyaline appearing substance. The epithelium of the parenchyma took the nuclear stain fairly well. Occasionally small areas were observed not having any nuclear stain at all. The amount of interstitial connective tissue was unusual; its capillaries were dilated and full of red blood-cells; it contained numerous nuclei and small hæmorrhagic spots; number of fat-cells considerable. A section made through the constricted part of the pancreas mentioned showed two spots of fat-necrosis. The capsule was thick, consisting of fibrous tissue, with numerous round, oval and spindle-shaped nuclei and hæmorrhagic areas. The development of fibrous tissue was greatest over the constricted portion.

Numerous bacilli, small and large, often forming strings, with square ends, were found in the capsule, extending to a less degree into the interstitial tissue between the acini for a short distance from the capsule.

Microscopic examination of the duodenum showed very large numbers of goblet-cells in the epithelial lining, moderate congestion, no cell-infiltration.

Case III. Although the fat-necrosis was not very extensive, on account of the interesting history and clinical features, it seemed best to consider this in connection with Cases I and II.

"The patient, a widow, sixty-six years old, had cholecystitis six years ago, and again, four years ago, this time with severe hepatic colic and jaundice. She made slow improvement and sustained a relapse six months later. There was no vomiting or nausea following this, and she slowly improved in most respects until late last autumn. She had been a stout woman, and now regained most of the weight that she had previously lost.

"There was no recurrence of jaundice nor was there evidence of troubled digestion, but during these years she suffered from frequent attacks of pain in the hepatic region. The pain was never severe enough to require anodyne, although hot applications were often made, and she felt that she was not entirely well. About the middle of October, 1899, she fell and sustained a slight strain of the body, after which the pains became worse. About the first of December, although the pain had recently somewhat diminished, she became slowly jaundiced, suffered from much gastric distress, with stools at times clay-colored, at other times showing some bile staining; the urine became beer-colored, and there was progressive loss of weight.

"She was examined by Dr. Stockton on the 20th of January, 1900, at which time she complained of dull pain in the small of the back, and occasionally in the hepatic region. She had moderate jaundice, clay-colored stools and fair gastric digestion. There was total acidity of 30, combined chlorides 10, acid salts 5, and free hydrochloric acid .05. There was no gastric catarrh, and the food showed good digestion. The urine was acid, dark reddish color, specific gravity 1023, showing a trace of albumin, bile coloring-matter, no sugar, a trace of

indican, the total amount in twenty-four hours 480 cc. with 7.84 grams of urea. Microscopically there were found a few bile-stained, finely granular casts, squamous epithelium, leucocytes and cylindroids. The diagnosis of cholecystitis and cholelithiasis was made. She was judged not to be in a condition suitable for operation, and was therefore sent to the Buffalo General Hospital to be treated by vapor baths, limited diet, hot fomentations, and large draughts of water. Under this treatment she made moderate improvement in most respects and more especially in the condition of the urine. The casts disappeared, as did the biliary coloring-matter, with the subsidence of the jaundice.

"On the 14th of February she was allowed to sit up and walk a little about the room. Immediately afterwards her temperature rose to 101° F., the pulse to 100, and at the same time there was an increase of pain and a return of the jaundice. An operation was now resolved upon. This was performed on the 21st of February by Dr. Roswell Park, which he has kindly described as follows:

"'After opening the abdomen by an S-shaped incision, the gall-bladder was found extremely contracted and drawn up beneath and behind the anterior border of the liver. In it could be felt at least one calculus, while along the duct others could be felt. The operation was made extremely difficult by the fleshiness of the patient, and by adhesions, as well as by the deep situation of the gall-bladder and the duct. During the manipulation required for making access to these, especially to the latter, I was perfectly conscious that more or less of damage was being done to adjacent structures, but was absolutely unable to estimate it. One calculus was removed entire from the gall-bladder, while most of the material in the duct had to be crushed before it could be removed with a spoon. The impacted stone which seemed to be producing complete obstruction of the duct was at least half an inch in diameter. Several small calculi were also removed. The material thus removed weighed 4 grams. Sutures were applied as carefully

and as accurately as was possible under the difficulties, and a small drain inserted into the gall-bladder.'

"The patient bore the operation well, but soon afterwards required stimulants. During the afternoon and evening she suffered severe pain, requiring anodyne. Early in the morning there suddenly developed a condition of shock, the pulse being 140 and thready, temperature being 102°. The patient was given an intra-venous injection of normal salt solution, hot enemata and hypodermic stimulation, after which there was temporary improvement, but death ensued about 3 P. M., twenty-eight hours after the operation.

"A clinical diagnosis of septic peritonitis was made, but this did not seem sufficiently extensive to account for the sudden development of the shock, and the early fatal termination of the case."

Report of Autopsy: February 22, 1900, two hours after death. The subject was a rather stout, middle-aged woman. There was an incision 12 cm. long in the right hypochondriac region, approximately parallel with the costal margin. The end of a rubber drainage-tube projected between the edges of the incision, which was closed with silkworm-gut sutures. The whole was covered with a bandage and dressing. The subcutaneous adipose tissue was 1½ cm. in thickness over the thorax, and 3 cm. over the abdomen. There was some arteriosclerosis and calcification of both coronary arteries; otherwise the heart and pericardium, as well as the pleural cavities and lungs, were not remarkable. The omental and mesenteric adipose tissues were well developed. The peritoneal cavity contained a quantity of dark, serous fluid. The peritoneal surfaces of the intestines and liver were covered with a thin layer of fibrin. The coils of intestines were congested. The spleen was more than twice its normal size, dark and soft. The kidneys, ureters and suprarenal bodies were not remarkable. The mucous surfaces of the stomach, the duodenum, and the remainder of the intestines, with the vermiform appendix, presented nothing noteworthy, except a small, clean-cut [illegible]

the pylorus. There was a firm fibrous adhesion between the lower anterior margin of the liver and the anterior abdominal wall above the umbilicus. The liver was of about the normal size, and was hard; its capsule thickened; its surface coarsely granular, in color pale and yellowish; the lobules distinct on section. The drainage-tube above mentioned entered the gall-bladder which was contracted, and passed through the cystic into the common bile-duct, ending 2 cm. from the bile-papilla. The lower end of the tube was secured to the wall of the duct by a purse-string silkworm-gut suture. The bile-papilla was prominent. The submucous layer of the duodenum in this region was thickened and red from infiltration with blood.

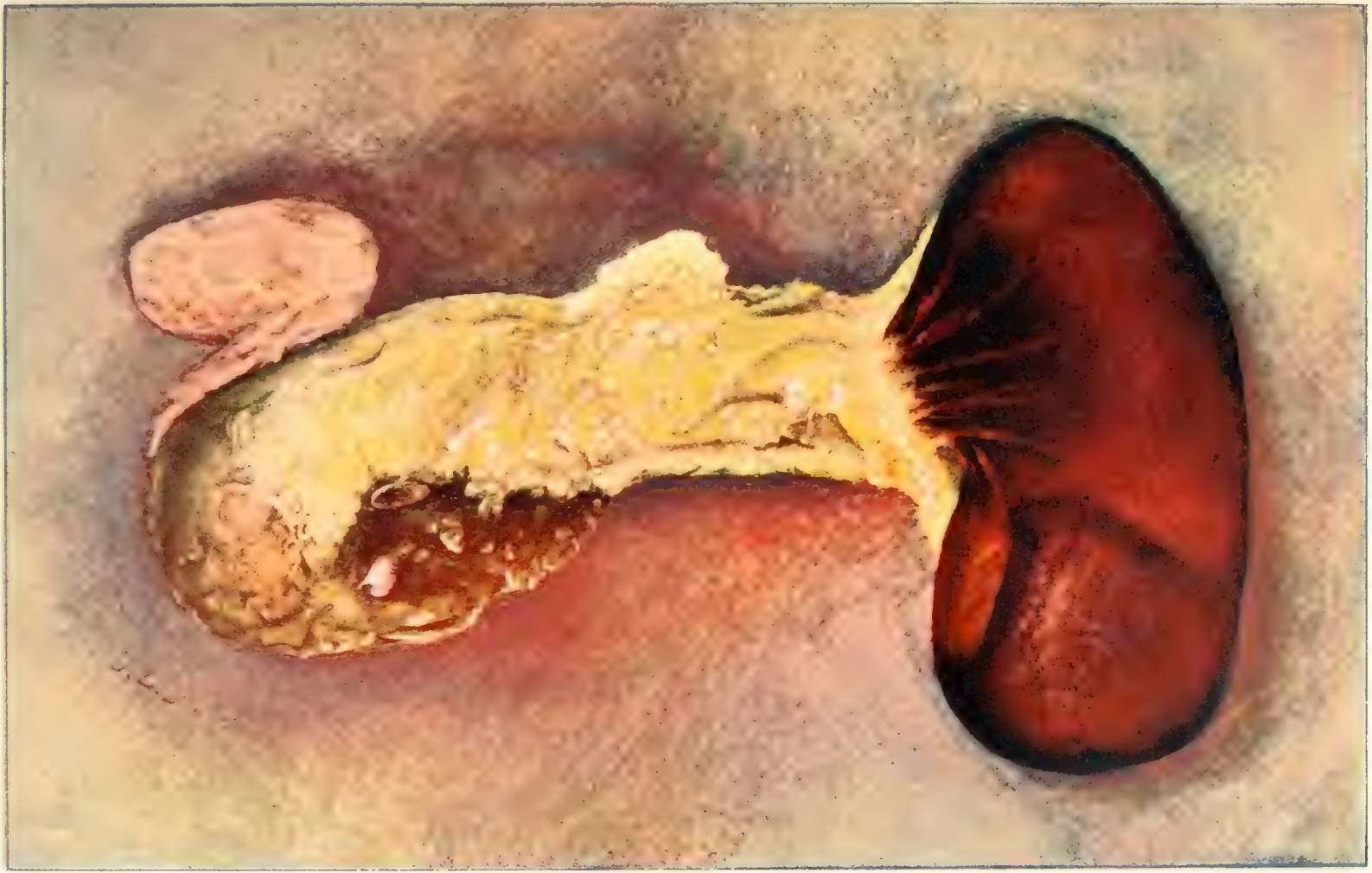
The pancreas was 18 cm. long, and showed well-marked fatty infiltration. The pancreatic duct was easily found, opening inside the bile-papilla, and was large and patulous. The head of the pancreas was enlarged; it was firm, and dark reddish-brown in color. On section it showed irregular, chalky-white areas, alternating with dark areas, apparently of hæmorrhagic infiltration. Incision into the body of the pancreas near its middle showed some small hæmorrhages in this vicinity. On both surfaces of the pancreas, but especially the posterior, there appeared numerous round, flat, firm, opaque white areas 1 to 5 mm. in diameter, plainly seen in contrast with the normal adipose tissue. They were regarded as fat-necroses, which they proved to be on examination with the microscope. (Plate 1.)

The lymph-nodes in the neighborhood of the pancreas were slightly enlarged, firm, and pink in color. It was, unfortunately, not possible to examine the solar plexus, as the time allowed for the autopsy was limited.

The mucous membrane of the duodenum showed nothing remarkable in its histology. The capillaries of the submucous layer close to the pancreas were distended with blood. The lymph-spaces of this layer contained numerous red blood-cells, and in some places were crowded with them. There were no large hæmorrhages.

Plate I.

Pancreas of Case III, showing numerous small fat-necroses in the adipose tissue about it; the head of the pancreas is somewhat enlarged, and is dark brown in color from hæmorrhage; part of the stomach, just entering the duodenum, appears above and behind the head of the pancreas; the spleen and the body of the pancreas have been given one half turn, so that their posterior surfaces are shown, and the upper border of the spleen appears in the sketch as the lower.



PANCREAS AND SPLEEN. CASE III.

The pancreatic duct and its branches possessed an intact epithelial lining. The ducts were usually empty; sometimes they held stringy contents, probably mucus. Sections of the pancreas showed fatty infiltration, and rather more abundant connective tissue, than is usual, in which "mast-cells" and a few plasma-cells (Unna) were seen. In osmic acid specimens slight fatty degeneration of the glandular epithelium became evident. Areas of fat-necrosis were numerous in the interlobular adipose tissue. Leucocytes occurred in the stroma of the pancreas, between the groups of acini, in moderate numbers, both in the body and in the head of the pancreas. There were points on the surface of the head where masses of leucocytes and fibrin, with much granular detritus, were seen. Numerous hæmorrhagic areas appeared in and about the head of the pancreas. Small hæmorrhages occurred in the body of the pancreas. Rarely small veins containing recent thrombi were observed. The dark color of the head of the pancreas was due partly to necrotic fat-cells whose contents were stained orange-red, apparently from the imbibition of blood coloring-matter. Masses of decolorized blood-corpuscles were also noted. Occasionally the necrotic fat-cells contained granules and rod-shaped crystals of brown pigment, and a similar pigment occurred between the fat-cells.

In the head of the pancreas numerous ill-defined areas occurred, in which the epithelial cells were shrunken, their nuclei staining badly. There were other areas in which the outlines of the epithelial cells were indistinct, and the nuclei took no stain at all. These changes were not marked. They were often seen in close contact with necrotic fat-cells.

The histological examination of the other organs of the body yielded no results appearing to have any relation with the condition in the pancreas.

Conclusions: There was no evidence of any obstruction in the pancreatic duct or any of its branches. Acute interstitial inflammation of the pancreas, with hæmorrhage, was present, but not in a marked degree. The other lesions of the pancreas were fat-necrosis in the surrounding and interlobular adipose

tissue, and necrosis of some parenchymatous cells. In the opinion of the writer, the pathological conditions are best explained as having resulted from traumatism to the pancreas sustained at the operation, and unavoidable on account of its difficulties. (See Dr. Park's description of the operation, page 13.) That such traumatism occurred is indicated by the hæmorrhages in the head of the pancreas, and in the adjacent submucous layer of the duodenum. The lower end of the drainage-tube in the common duct was only 2 cm. distant. A disturbance of the tissues of the head of the pancreas would account for the necrosis of the parenchymatous cells, and would make escape of the pancreatic ferments possible. Fitz[1] has reported a case in which pancreatitis and fat-necrosis resulted from an injury. The significance of the pigment crystals in the head of the pancreas, and partly inside of necrotic fat-cells, is not clear. They must have been due to imbibition of blood or bile coloring-matter, perhaps, though not probably, from an earlier lesion; chemical tests gave indecisive results. It is possible that the beginning of the affection coincided with the rise of temperature and pulse noted in the clinical history as occurring a week before the operation. That is unlikely, however, as none of the classical symptoms of pancreatitis were present at that time.

HISTOLOGY OF THE FAT-NECROSES

The histological appearances of the necrotic fat-material were identical in Cases I and II; they will therefore be described together. The pultaceous fluid consisted of opaque masses and granules, highly refracting globules, and needle-shaped crystals. Frozen sections of the white necrotic nodules showed them to be made up of similar coarse granules and masses, globules and crystals, and a small amount of masses of brown pigment. Many of the opaque masses were about the size and shape of fat-cells and evidently represented altered fat-cells. (Plate II, Fig. 1.) The surrounding tissue was moderately congested; a few small extravasations were noted. In

fat-cells appeared normal. Sections of the same imbedded in celloidin and stained with hæmotoxylin or carmine gave similar results. In them, however, the presence of a connective tissue boundary to the spots of fat-necrosis was often demonstrated, even to the extent of forming a thick capsule. There was marked infiltration of this capsule and less of the underlying part, with cells having good-sized, round, oval or spindle-shaped nuclei. The relatively healthy fat-tissue sometimes showed an increased number of nuclei. (Plate II, Fig. 2.) Furthermore, owing to the partial solution of the opaque material by the ether used in imbedding, the outlines of what appeared to be fat-cells could be plainly seen in parts of these necrotic areas; and strands of connective tissue were visible traversing the areas. The nuclei of the altered fat-cells could not be stained. Frozen sections of the spots of fat-necrosis were partially cleared up by the use of either ether or acetic acid; when the two reagents were used successively, the opacity disappeared entirely, more readily if the preparation was heated moderately. Calcium was found to be present in abundance. According to the observations of Langerhans,[21] the calcium is in combination with fatty acids. Sarfert[31] has reported finding the fatty acids combined with sodium.

The prominence of the nodules of fat-necrosis and the condensation of the connective tissue in front of them point toward an increase in size. It has already been noted that relatively unaltered fat-cells and strands of connective tissue were to be seen within them. The congestion, hæmorrhages and cell-infiltration indicate an inflammatory reaction about them.[3] Many sections appear to justify the view of Langerhans that the connective tissue boundary of the necrotic nodule coincides with the connective tissue originally surrounding a fat-tissue lobule.

The nodules of necrotic adipose tissue in Case III were confined to the vicinity of the pancreas. They were smaller, more flattened, and not so sharply defined as in the other two cases. No thickening of the connective tissue about them was seen. The meshes of the adipose tissue contained a somewhat

Plate II.

Fig. 1. Edge of an area of fat-necrosis, normal adipose tissue at the right. Frozen section; photo-micrograph.

Fig. 2. Edge of an area of fat-necrosis, showing cellular connective tissue surrounding it, and normal adipose tissue at the right. Celloidin section; hæmatoxylin; photo-micrograph.

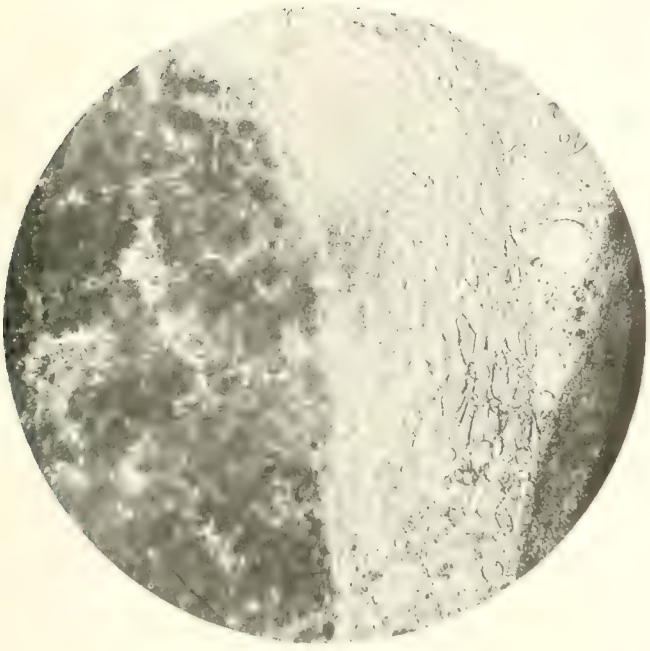


FIG. 1.

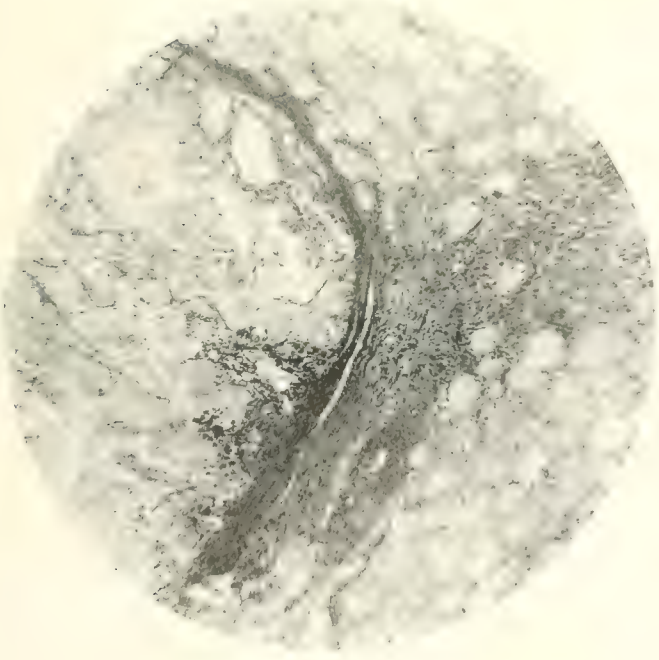


FIG. 2.

increased number of leucocytes and larger mononuclear cells, as well as small hæmorrhagic areas. None of these features were more noticeable directly about the nodules. The outlines of the fat-cells in the necrotic nodules were entirely preserved, and crystalline needles were easily seen in sections mounted in balsam. These needles sometimes stained blue with hæmatoxylin. They frequently appeared to radiate from a point in the centre of the cell, and often were arranged thickly and in a radiating arrangement about the circumference of the cell, leaving a cavity in the middle. The histological characters were, in the main, identical with those described hereafter for fat-necroses in the hog and cat.

Calcium salts were also abundant in the nodules in Case III, which is interesting considering the rapid progress of the case. (Compare with the result in cat 37 described hereafter.

HISTOLOGICAL TECHNIQUE.

For fixation of the necrotic adipose tissue and the specimens of pancreas, alcohol, formaldehyde (4 per cent. solution), Müller's fluid, Flemming's solution, and osmic acid were employed. Alcohol with celloidin imbedding, performed rapidly, gave the best results. The sections need not be very thin. Various aniline dyes and other stains were tried, but hæmatoxylin and eosin were found the most satisfactory. The contents of the necrotic nodules often stained deeply with hæmatoxylin, probably owing to the presence of calcium. The necrotic areas were not stained by osmic acid or Sudan III, although some oil globules which took the stain might be seen in them. Frozen sections, which are indispensable, may be made either from the fresh tissues or after fixation with formaldehyde. For the micro-chemical reactions of fat-necroses the paper of Langerhans[21] may be consulted.

BACTERIOLOGICAL EXAMINATIONS.

Numerous bacilli were found between the fat-cells about the spots of fat-necrosis in Case II.; large rods with square ends

were most numerous; some smaller forms with rounded ends were also seen. They were not detected in the spots of fat-necrosis themselves, nor were they more noticeable in their immediate vicinity than at some distance removed.

No bacteria could be found with the microscope in the fatty tissues of Case I., and unfortunately the circumstances attending the autopsy prevented the use of culture-methods. In Case II., however, inoculations were made both from the pancreas and the necrotic fat. From each a variety of bacillus was isolated in pure culture.

From the fat a bacillus was obtained, having rounded ends, growing both as short oval forms and as long filaments, actively motile, liquefying gelatine rapidly, and flourishing on the ordinary culture-media. It appeared to be a variety of Proteus. Broth-cultures injected into the abdominal subcutaneous tissues of rabbits evoked local suppuration; nothing resembling fat-necrosis was produced. From the pancreas a bacillus with rounded ends was isolated, growing both as short oval and long filamentary forms, not motile, or but slightly so, not liquefying gelatine, growing on the ordinary culture-media, coagulating milk, producing gas and acid reaction in 2 per cent. glucose-bouillon, giving a faint indol reaction in peptone solution, and in all respects behaving like Bacillus coli communis. Broth-cultures injected into the subcutaneous tissues of the abdominal wall of rabbits evoked local suppuration, but nothing like fat-necrosis could be found.

We do not appear, in either of these forms, to have secured the large bacillus with square ends seen in sections of the pancreas and fat-tissues. One is not, however, disposed to lay much stress upon it or upon them. It will be remembered that the pancreas lay, floating almost, in a cavity filled with a kind of pultaceous *débris*, and that this cavity was in part bounded by the splenic flexure of the colon, whose mucous membrane was in a necrotic condition. Under these circumstances the penetration of bacteria into the pancreas and fat to the extent observed is not surprising.

In case III., the bacteriological examination gave the follow-

ing result: The peritoneal fluid, the liver, and the pancreas contained an organism in pure culture, which, in regard to motility, staining by Gram's method, growth on gelatine and agar plates and tubes, growth on potato, coagulation of milk, fermentation of glucose and development of indol, corresponded with Bacillus coli communis. A tube made from the pancreas, and kept in the incubator under anaerobic conditions remained sterile. A tube from the spleen remained sterile. Sections of the pancreas stained in methylene-blue showed rarely small bacilli with rounded ends, exhibiting no special relation with the spots of fat-necrosis. Considering the conditions present in this case, no significance can be attached to infection with Bacillus coli communis.

MINOR FAT-NECROSES ABOUT THE HUMAN PANCREAS.

FREQUENCY OF OCCURRENCE.

In the paper by Balser[1], in which disseminated or multiple fat-necrosis was first named and accurately described, it was also remarked that one or a number of areas of fat-necrosis might often be found in the adipose tissue in or near the pancreas in the cases of individuals dying from causes not connected with the pancreas.

Judging from the remarks of Fitz[7] and others, the same appears to have been noted by many observers. Reports vary considerably as to its frequency. Balser[1] discovered it at five (20%) of twenty-five unselected autopsies; Langerhans[21] at twenty-eight autopsies, four times. (14.3%); Kasahara[18], only once (1.2%) in eighty-three autopsies. In Chiari's[4] studies on auto-digestion of the pancreas, that organ was examined at seventy-five autopsies, and fat-necrosis was seen incidentally twenty-three times, (30.6%). None of the writers who have described fat-necrosis of this form appear to have found it

associated frequently with obstruction of the duct of Wirsung or with any disease of the pancreas.

ANTE-MORTEM AND POST-MORTEM FAT-NECROSES

Langerhans[22] suggested that such fat-necroses might be due to the *post-mortem* action of the fat-splitting ferment of the pancreatic juice. This explanation accounts very plausibly for many of the cases in which no inflammatory reaction appeared in the tissues surrounding the altered fat-cells.

The writer has examined the pancreas with great care at one hundred consecutive autopsies, nearly all on adults, and found small areas of fat-necrosis eight times (8%). The affected areas were small, irregular in form, and close to the pancreas. Necrosis of the cells of the pancreas itself was many times seen directly adjacent to altered fat-cells. The changes in the pancreatic cells conformed to the auto-digestion of the pancreas described by Chiari[4], (see below). There was evidence that the process was not recent, (in the shape of inflammatory reaction of the surrounding tissue), only in two cases. In the six cases in which the fat-necrosis was recent or post-mortem, the pancreas was normal in all but one, where catarrhal inflammation, indurative pancreatitis, and small cysts of the pancreas were present. The two cases in which the fat-necrosis was evidently of longer standing are described below (cases A and B) with two others which have occurred recently in the writer's service (cases C and D). It will be observed that in each of these cases some form of pancreatic disease was present. In Case C, the changes were most extensive, and may possibly represent the late results of an earlier hæmorrhagic pancreatitis.

Case A. [illegible] County Hospital, March, 1892. [illegible], a woman, forty-five years of age, moderately fat, having gall-stones, suppurative cholangitis, acute nephritis; urine albuminous, with bile-stained, hyaline and waxy casts, and bile-stained renal epithelium. The pancreas was large, parenchyma [illegible] apparently [illegible]. Near the head was a round,

white area 3.4 m.m. in diameter, from which a white, semi-fluid material exuded on slight pressure. Examination of frozen sections showed it to be a region of fat-necrosis in the interstitial adipose tissue. Calcium was present in abundance. In stained sections the periphery of the nodule was found to be rich in cells with round and oval nuclei, and a few polynuclear leucocytes among them, readily shown in thin sections stained with hæmatoxylin. There was a thin band of dense connective tissue around the nodule. The epithelium of the parenchyma of the pancreas was fatty degenerated; there was an increased amount of interstitial tissue; a few ducts contained desquamated epithelium and leucocytes.

Case B.—Buffalo General Hospital, Nov. 18, 1898. W. P., male, age 48, with acute pleuritis, chronic endocarditis, hypertrophy and dilatation of the heart, cyanotic induration of liver, spleen and kidneys, ascites, catarrhal gastritis. The pancreas was small and firm, its duct dilated for the most part, but in places stenotic; the duct contained numerous small, white calculi. On section the parenchyma of the pancreas was found almost entirely wanting. A few islands of normal tissue remained, however, and some atrophic lobules, all of them imbedded in dense fibrous tissue. The whole was surrounded by adipose tissue, in which fat-necrosis was prominent, especially of the fat-cells close to the pancreas. Needle shaped crystals of brown pigment were seen in and between the altered fat-cells. The adjacent connective tissue contained numerous large mononuclear cells.

Case C.—Buffalo General Hospital, March 8, 1899. A. J., male, age 50. History of syphilis, alcoholism, and dementia, beginning six years before death. The autopsy revealed the presence of chronic leptomeningitis, cerebral œdema, encapsulated areas of caseation in the lungs, catarrhal gastro-enteritis, fatty and cirrhotic liver. The pancreas was small, indurated; the tail of the pancreas bound to the stomach by a firm adhesion. The duct of Wirsung could not be found. The common bile-duct appeared normal. There was abundant adipose tissue about the pancreas, in which numerous grayish, chalky

areas were seen, which proved to be fat-necroses, containing an abundance of calcium. On section a small number of pancreatic lobules were seen, enclosed in broad bands of fibrous tissue. Few ducts were visible, all similarly enclosed in fibrous tissue. The fibrous tissue constituted the bulk of the structure. It penetrated between the individual acini. It contained numerous large mononuclear cells and leucocytes, few plasma-cells, and a moderate number of mast-cells. There were numerous large cells enclosing yellow and brown pigment granules. These appeared to be phagocytic cells. The areas of fat-necrosis were encapsulated by fibrous tissue of the same character, and stained intensely with hæmatoxylin. The fat-necroses were larger and more numerous, and the changes in the pancreas more striking than in any of the others of this series.

Case D.—Buffalo General Hospital, Feb. 21, 1900. J. N., male, age 49, lobar pneumonia, horseshoe kidney. A small accessory pancreas occurred below the mucous membrane of the jejunum. The pancreas was large and firm, weight 143 grams, showing moderate fatty infiltration. Several small, yellowish-white nodules 2 to 4 m.m. in diameter occurred in the interlobular tissue. They proved to be fat-necroses, surrounded by fibrous tissue, rich in spindle-shaped cells. In one case a small vein close by contained a thrombus of leucocytes. The pancreatic ducts were filled with desquamated epithelium, leucocytes and granular material.

Auto-digestion of the Pancreas.

In this connection the auto-digestion of the pancreas described by Chiari[4], is of great interest. Chiari encountered two cases at autopsy, showing necrosis of the parenchyma of the pancreas with secondary formation of fibrous tissue. Having in mind the possibility that this necrosis might have been due to the action of pancreatic ferments, he examined the pancreas at seventy-five autopsies, finding that necrosis of the acini was present only in thirty-five, which were not, in the main, exam-

ined sooner after death than the other cases. In the forty cases in which necrosis of the acini was present, it either involved nearly the whole pancreas, or was localized and scattered. The nuclei of the epithelial cells of the necrotic acini refused to stain, and the protoplasm of these cells had a homogeneous appearance. The interstitial tissue also was sometimes necrotic. In the absence of any other cause, it seemed probable that these necroses were due to the "agonal" or post-mortem action of the ferments of the pancreas. In seven cases the necrosis was supposed to be "agonal," on account of being accompanied by some extravasation of blood. No relation to any closure of the duct of Wirsung was demonstrated, and no reason was discovered for the presence of the condition in about one-half of the cases, and its absence in the other half.

In the course of his examinations of the pancreas, the writer has frequently seen a condition similar to that described by Chiari. He has several times observed that groups of acini whose cells failed to take a nuclear stain, occurred close to fat-cells which were opaque from the presence of granules and crystals. Evidently there is a close analogy between the alteration of protoplasm by certain pancreatic ferments as maintained by Chiari, and the decomposition of the fats in fat-cells, probably by the fat-splitting ferment.

Chiari's observations have important bearings with relation to the genesis of pancreatic cysts and to the frequency of hæmorrhages in the pancreas, which cannot be discussed here. It was long ago suggested by Klebs (quoted by Chiari), that a corrosive action of the pancreatic juice might be the cause of pancreatic hæmorrhages.

Several writers have called attention to the resemblance of these phenomena to the part played by the gastric juice in producing peptic ulcer of the stomach and auto-digestion of the wall of the stomach. The alterations seen in certain cells of the gastric glands when the tissues are not fresh, well known to histologists, are doubtless of the same character.

FAT NECROSIS IN THE PANCREAS OF THE HOG.

Balser[2], who was the first to describe fat necrosis in the human subject accurately, was also the first to study its appearance in the hog in detail. He found it in the fat-tissue in or about the pancreas in nearly all Hungarian swine, frequently in Algerian, and in a few German swine. He found in the necrotic nodules bodies resembling the fungus of actinomycosis. In order to determine whether or not fat-necrosis occurred in American hogs the writer examined the pancreas in one hundred hogs. It was impossible to learn anything more concerning the animals than that they were raised either in Ohio, Indiana, Illinois or Michigan, and were apparently sound and healthy. The pancreas of the hog is surrounded by a quantity of adipose tissue, which is also abundant between the lobules. The fat-cells are very large. Little white flecks consisting of single fat-cells or groups of fat-cells are often seen in the pink parenchyma. Not rarely one meets with dark red spots in the parenchyma, several millimetres in diameter, apparently the result of hæmorrhage. The organ, taken as fresh as possible, was cut in slices one to three millimetres thick, and each slice carefully examined. In two cases fat-necrosis was found.

In one of these the number of areas of fat-necrosis was not large and they were confined to a limited region. In the other they were numerous and were scattered throughout the organ. They were not conspicuous, being distinguished by their more yellow color, which contrasted with the white, normal fat. They appeared much like minute abscesses, but were somewhat harder than the normal fat. In shape they were irregular. Their various dimensions were one to two millimetres only. They nearly always impinged on one side against a portion of the parenchyma. Frozen sections showed them to be made of fat-cells, rendered opaque by the presence of numerous needle-shaped crystals, frequently arranged in the form of a ring about the circumference of the cell. They contained an abund-

ance of calcium. Sections stained in hæmatoxylin and eosin gave the appearances already described. (Plate III, Fig. 4.) The contents of the areas exhibited usually a strong blue stain. The borders were surrounded by a band of connective tissue with numerous connective tissue cells, and a small number of polynuclear leucocytes. Leucocytes also occurred in small numbers among the cells of the necrotic areas. Often they were in rounded clumps, corresponding to the outlines of a fat-cell, and suggesting that they might have migrated into the interior of a necrotic cell. The ray-fungus described by Balser after using the Ehrlich-Biondi stain was not found; nor were the hæmorrhages about the necrotic areas. Examination of sections for bacteria was negative. Cultures were not made. The parenchyma of the pancreas was not remarkable. None of the other organs were examined.

EXPERIMENTAL FAT-NECROSIS.

The relation existing between fat-necrosis and affections of the pancreas, and especially pancreatitis, has been demonstrated by Fitz[7], and has been illustrated by the cases reported above. As has already been stated, the essential change in fat-necrosis is a decomposition of the neutral fat molecule into its component fatty acid and glycerine. Frequently, if not always, the fatty acid unites with calcium to form a new compound, which is a soap. It is remarkable that the fat-splitting ferment of the pancreas accomplishes this very decomposition of neutral fats. Certain experiments reported within the last few years seem to show the exact nature of the connection between fat-necrosis and affections of the pancreas.

Hlava[15] excited a hæmorrhagic pancreatitis with fat-necrosis in a cat by injecting the diphtheria bacillus into the pancreas after laparotomy.

Langerhans[22] suggested that the decomposition of neutral fats which occurs in this process might be accomplished by the fat-splitting ferment of the pancreas. He announced that

Plate III.

Fig. 1. Cat 8. Fat-necrosis in the perirenal adipose tissue showing the outlines of the affected cells faintly, and cell-infiltration about the necrotic area. Celloidin section; borax carmine; low power; photo-micrograph.

Fig. 2. Cat 22. Fat-necrosis in the omentum, showing the opacity of the area, and the outlines of the altered cells in it; frozen section; low power; photo-micrograph.

Fig. 3. Spontaneous fat-necrosis in the omentum of a cat, celloidin section; hæmatoxylin and eosin; × 80; photo-micrograph.

Fig. 4. Hog. Small area of fat-necrosis close to the pancreas; celloidin section; hæmatoxylin and eosin; × 80; photo-micrograph.

FIG. 1.

FIG. 2.

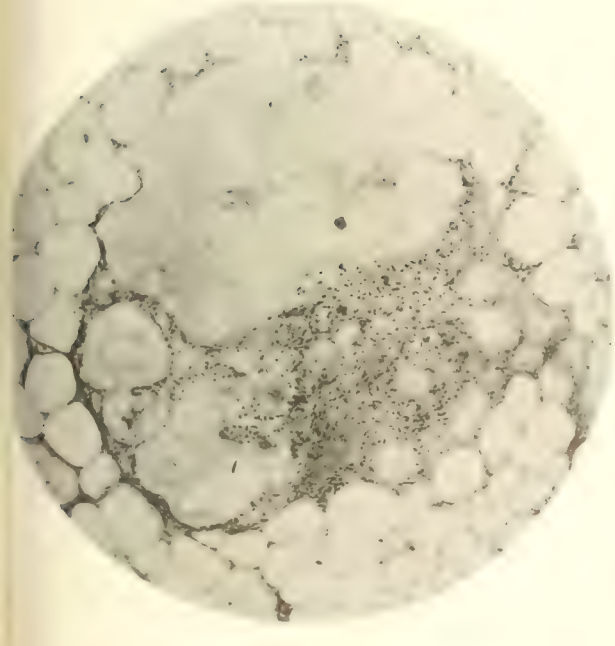


FIG. 3.

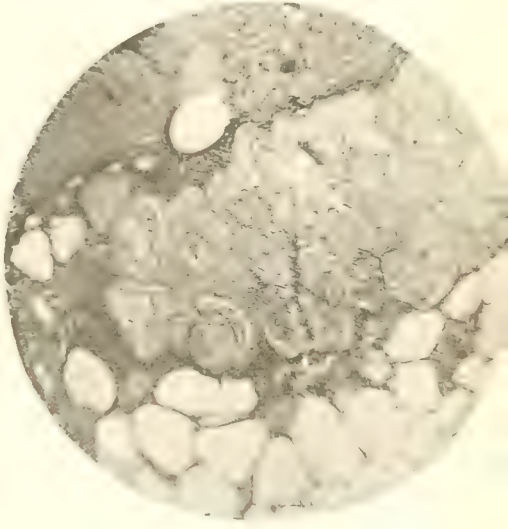


FIG. 4.

he had succeeded in producing fat-necrosis in a rabbit. This was the only positive result among twelve animals (rabbits and dogs) experimented upon. He made use of a suspension of rabbit's pancreas in distilled water, which was injected into the adipose tissues. His positive experiment is open to the objection that bacterial contamination was not excluded with certainty.

Dr. Whitney, of the Harvard Medical School, allows me to state that he ligated the pancreas in a number of dogs and produced fat-necrosis in one of them. No detailed account of his work has been published.

Hildebrand[14], and his student, Dettmer[5], placed a ligature about the gastro-splenic portion of the pancreas, to prevent the discharge of its secretion through the duct, in two cats; and in six others they performed the same operation and also ligated the veins leaving the organ. In all cases fat-necrosis was found about the pancreas. They were also successful in producing it three times by introducing portions of the pancreas of one cat into the abdominal cavity of another. In one instance they obtained it after removing a piece from the organ and leaving the distal portion without ligature. Hildebrand, furthermore, injected pure trypsin into the abdominal cavity, and found that hæmorrhages into the peritoneum resulted. He suggested that the hæmorrhages, so frequent in pancreatic affections, might be due to the action of trypsin, while the fat-splitting ferment was responsible for fat-necrosis.

Hildebrand and Milisch, Flexner[9], Katz and Winkler[19], Opie[23] and others have continued and enlarged upon these experiments with confirmatory results.

Rosenbach, and his pupil Jung[17], with a similar object in view, introduced trypsin and at other times portions of pancreas, into the abdominal cavities of rabbits. Out of four trials with pieces of pancreas, they obtained fat-necrosis once, using dog's pancreas.

LIGATION AND INJURIES OF THE PANCREAS.

As the work of Hildebrand seemed to have been the most productive of results, the writer first attempted to verify his conclusions.* The animals selected were two dogs, one rabbit, and seventeen cats. They were anaesthetized with ether. The operations consisted in the placing of a ligature about the gastro-splenic portion of the pancreas near the duodenum, or the duodenal portion of the pancreas, or both. In most cases as many as possible of the veins leaving the pancreas were also tied. In the majority of cases the pancreas was lacerated with a sharp hook. In one instance the gastro-splenic portion of the pancreas was cut through on the distal side of the ligature. An aseptic technique was followed, and the results were good with the exceptions noted below. The peritoneum and skin were closed separately with silk sutures. The wounds in the abdominal walls frequently furnished slight purulent discharges. Owing to the freedom allowed to the animals it was found impossible to secure primary union in every instance. Animals that died during the first twenty-four hours after operation were not included in this report.

The operations and their results in detail were as follows:

Dog. Weight 7 lbs., 1½ oz. A silk ligature was tied about the gastro-splenic portion of the pancreas. The animal, while apparently in perfect health, was killed at the end of one week; weight 8 lbs., 6½ oz. There was no peritonitis and no fat-necrosis.

Dog. Large, fairly nourished. The gastro-splenic portion of the pancreas was ligated. The dog was killed after ten days, somewhat emaciated. There were adhesions and suppuration about the ligature, but no general peritonitis. There was no fat-necrosis.

Rabbit. Moderately fat. The gastro-splenic portion of the pancreas was ligated. The animal remained in good condi-

tion, and was killed after three weeks. There was no peritonitis. There were traces of fat-necrosis close to the ligature.

From the seventeen cats there were ten negative results. In two there were minute areas probably of fat-necrosis. In five there was well-developed fat-necrosis. One of the five exhibited disseminated fat-necrosis. In detail, the operations and results were as follows, the order in which the cases are arranged being based on the time which elapsed between the operation and the autopsy :

Cat 11. Weight 6 lbs., 14 oz. The gastro-splenic portion of the pancreas was ligated. The animal was killed at the end of one week; weight 6 lbs., 5 oz. There was no fat-necrosis nor peritonitis.

Cat 8. Weight 4 lbs., 1 oz., but fat. The gastro-splenic portion of the pancreas was ligated. The animal, while apparently healthy, was killed after one week; weight 4 lbs., 2½ oz. There was no peritonitis. Small nodules of fat-necrosis were discovered in the interlobular pancreatic fat and in the omental fat. There was one nodule in the perirenal fat (Plate III, Fig. 1), and one in the mesorectum. No bacteria could be demonstrated by staining methods in or near these areas.

Cat 6. Weight 7 lbs., 10 oz., well nourished. The gastro-splenic portion of the pancreas was ligated. The cat was killed after two weeks; weight 6 lbs., 13 oz. There was no fat-necrosis nor peritonitis.

Cat 7. Weight 7 lbs., 4 oz., fat. The gastro-splenic portion of the pancreas was ligated. The cat was killed after three weeks; weight 5 lbs., 13½ oz. A few minute spots, probably of fat-necrosis, were found in the omental fat. Staining methods showed no bacteria in or about them. There was no peritonitis. This was one of the two cases classed as doubtful.

Cat 4. Weight 4 lbs., 8½ oz., poorly nourished. The gastro-splenic portion of the pancreas was ligated. The cat remaining healthy was killed after four weeks; weight 4 lbs., 2½ oz. There was no fat-necrosis nor peritonitis.

Cat 3. Weight 6 lbs., 3 oz., fat. The duodenal portion of the pancreas was ligated. The cat was killed after one week,

then seeming to be in good condition. There was no peritonitis. Traces of fat-necrosis appeared close to the ligature. This was the second of the two cases classed as doubtful.

Cat 13. Weight 5 lbs., 15½ oz. Both the gastro-splenic and the duodenal portions of the pancreas were ligated, and as many as possible of the veins leaving the gastro-splenic portion were tied, and the continuity of the tissue of the pancreas was broken by a sharp hook passed into it beneath the peritoneum. The animal died after four days, being much emaciated. The autopsy was unavoidably delayed until about thirty-six hours after death. There was no peritonitis. There were several thin, flat areas of fat-necrosis on the surface of the omentum. The mucous membrane of the pyloric end of the stomach showed a number of small round excavations having all the characteristics of peptic ulcers. All the peritoneal surfaces were abundantly covered with large bacilli, stained by Gram's method. No relation in their distribution to the spots of fat-necrosis could be made out. Similar bacilli were present in large numbers on the mucous membrane of the stomach and duodenum, extending into the subperitoneal tissues and about the pancreas. In view of the long time that elapsed before the autopsy was made they were not regarded as of importance.

Cat 18. Both the gastro splenic and the duodenal portions of the pancreas were surrounded with ligatures, as well as the veins leaving the former. The cat died after forty-eight hours. There was no fat-necrosis nor peritonitis.

Cat 19. The operation and result were similar to those in Cat 18.

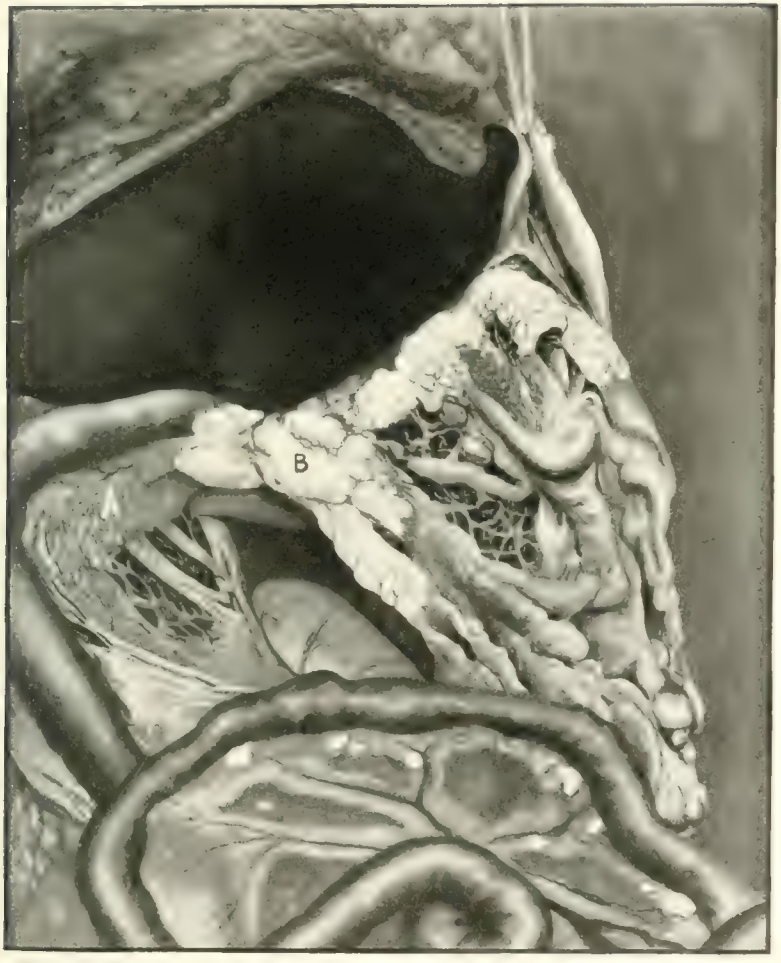
Cat 21. Weight 4 lbs., ½ oz. The gastro-splenic portion of the pancreas was ligated and as many as possible of the veins issuing from it, and the substance of the organ was broken with a hook. The animal died after four days; weight 3 lbs., 11½ oz. The autopsy was made in less than twenty-four hours. The peritoneal surfaces were covered with a fibrinous exudate. Beneath the peritoneal exudate a few thin, flat, white areas were seen, which proved to be necrotic adipose tissue. The

exudate contained great numbers of diplococci and fewer large bacilli, both staining by Gram's method. The diplococci alone were recovered in cultures. They grew feebly, not liquefying gelatine, and very quickly died out. In sections, no grouping of the micro-organisms with reference to the spots of fat-necrosis could be demonstrated.

Cat 22. Weight 4 lbs., 13½ oz., not fat. The gastro-splenic portion of the pancreas and the veins leaving it were ligated, and its substance was injured as in the other cases. The wound in the abdominal wall suppurated. The cat was killed on the fifth day after the operation; weight 4 lbs. At the autopsy, which was made immediately, the writer was astonished to find in the abdominal cavity a condition of disseminated fat-necrosis comparable to that occurring in man. (Plate IV.) In the vicinity of the pancreas, the retroperitoneal and omental fat was swollen, white and opaque, over an extent reaching twelve millimetres or more from the pancreas, and radiating from it in irregularly shaped masses. Smaller areas of similar character were scattered in great numbers through the omental fat and that of the mesentery along its entire length, even to the mesorectum. They occurred also in the perirenal fat. There was no peritoneal exudate. The animal was nevertheless the subject of an infection, proceeding, no doubt, from the wound in the abdominal wall. The pus in this wound contained a variety of organisms, among them diplococci and large bacilli. Although examination of coverslips was negative, cultures from the spots of fat-necrosis and from the liver yielded diplococci or short streptococci, growing at room temperature, not liquefying gelatine, forming minute white circular colonies on agar, and staining by Gram's method. Cultures from the pancreas and the blood of the right heart were negative. The distribution of the necrotic spots, especially of those in the mesentery, made them appear suspiciously like minute abscesses, but the microscope revealed their true character; nevertheless their arrangement was very suggestive of embolism. In a section of the mesentery, diplococci were found in and about one of the areas of fat-necrosis, and also, in

Plate IV.

Disseminated fat-necrosis in Cat 22 following ligation of the pancreas. General appearance of the abdominal cavity, seen partly from the right side. A is the pancreas, B is placed on the large masses of necrotic adipose tissue in the omentum. Numerous small localized fat-necroses are seen in the mesentery.


B

smaller numbers, at points in the mesentery remote from it. Otherwise, the examinations of sections of the necrotic areas for bacteria were negative.

Cats 27, 29, 31, 32 and 36 were good-sized and healthy. In each case the gastro-splenic portion of the pancreas was constricted by a ligature near the duodenum, as many as possible of the veins leaving it were tied, and the tissue of the pancreas was lacerated as in the other cases. The animals all made good recoveries. Their abdomens were opened at periods varying from eight to seventeen days. There was no peritonitis, and no fat-necrosis in any case.

Cat 34. Large and rather fat. The gastro-splenic portion of the pancreas was ligated near the duodenum, and the pancreas was then cut completely through on the distal side of the ligature, with the intention of allowing its secretion to flow into the peritoneal cavity. The animal died at the end of a week. The autopsy was made eighteen hours after death. The peritoneal sutures were found to have given way. The abdominal cavity contained a quantity of pinkish-white turbid fluid. The peritoneal surfaces were congested and covered with a thick layer of fibrin. The adipose tissue of the omentum appeared swollen and œdematous. Irregular white patches of necrotic adipose tissue were seen in the omentum and in the vicinity of the gastro-splenic portion of the pancreas, especially near the cut extremity. Smears made from the peritoneal exudate showed it to contain many large bacilli, and minute cocci. Cultures made from the peritoneum, the viscera, and the blood gave only minute white colonies of diplococci, staining by Gram's method, lancet-shaped and like the diplococcus of pneumonia in form. Sections of the necrotic adipose tissue and of the pancreas exhibited great numbers of large bacilli and diplococci, by the Gram-Weigert method, mostly on the surfaces, and not showing any special relation to the areas of fat-necrosis. Sections of the pancreas demonstrated necrosis of the fat-cells about it and in the interlobular septa in a striking manner.

Histology of the Fat-Necroses.—In all of the animals studied,

the diagnoses were based on the microscopic examination of the tissues, which is indispensable. Many pictures of great beauty illustrating the condition were obtained. The tissues hardened in alcohol were found to be very well adapted to histological purposes. A four per cent. solution of formaldehyde preserved the macroscopic appearances more satisfactorily. One per cent. osmic acid solution makes an excellent hardening agent, as the necrotic areas are not stained black by osmic acid. The areas of fat-necrosis were sometimes rounded and nodular, about one to two millimetres in diameter; sometimes they were broad and thin. They occurred exclusively in the fat adjacent to the peritoneum, usually in immediate contact with it. A disposition of the necrotic process to affect the vicinity of the ligatures about the pancreas or the veins was noted, but it was not constant or very marked. In recently killed animals the recognition of the areas was easy, owing to their opacity, contrasting with the relatively transparent normal fat; when a peritoneal exudate was present, it was often difficult to recognize them beneath the layer of fibrin.

In frozen sections the areas were opaque, contrasting with the neighboring fat. (Plate III, Fig. 2.) A brown tinge was often visible. Such complete disorganization of the adipose tissue as occurs in the human subject was in no instance observed. The areas consisted of rounded bodies similar in outline to the fat-cells. In most cases the contents of these bodies were minute needle-shaped crystals. Frequently the crystals were arranged in a radial manner at the circumference of the circle. The central portion was then empty or contained oil droplets. Such bodies in transverse sections appeared as rings. (Plate III, Fig. 4.) Calcium salts were demonstrated in some nodules in abundance. The contents of the necrotic cells in balsam preparations usually appeared homogeneous, although their crystalline nature was sometimes discernible. They reacted variously with stains, in some instances showing an affinity for eosin, in others staining deep blue with hæmatoxylin. The more intense hæmatoxylin stain

noted in some nodules, the writer supposes, to have been due to an abundance of calcium salts. The nuclei of the necrotic cells could not be identified. The amount of cell-infiltration about the nodules of fat-necrosis was quite variable. In some examples it was very slight, more often it formed a distinct band about the circumference where it was mingled with granules most of which stained deeply with nuclear dyes. Frequently it passed widely into the surrounding tissues. The cells were in part polynuclear leucocytes. In part they were larger, mononuclear elements rounded or spindle-shaped in form, with round or oval nuclei. They generally were of moderate size, though quite variable in this respect. Fragmentation of nuclei was a prominent feature in this zone, the fragmentation being most extensive in the region immediately adjacent to the necrotic fat.

In and around the areas of fat-necrosis numerous granules and rounded masses occurred, staining with carmine, hæmatoxylin, and by the Gram-Weigert method. Some of these were evidently fragmented nuclei; others were hyaline in character; others, which stained very deeply with hæmatoxylin, were supposed to contain calcium. These granules were often so numerous as to obscure the nuclei at the margin of the area. They rendered the search for bacteria in sections difficult and unsatisfactory.

The changes taking place in the pancreas were studied only with reference to the question in hand, and the account of them must be brief. The ligatures about the pancreas were found enclosed in a zone of leucocytes or good-sized mononuclear cells, or both, and often also of fibrous tissue. The cell-infiltration frequently passed into the interlobular connective tissue of the pancreas and over its surface. In the animals that were allowed to live longest there were marked atrophy and induration of the ligated extremity of the organ, which the microscopic examination showed to be due to an atrophy of the acini and an abundant formation of fibrous tissue between the lobules, and even between the acini. Desquamation of the epithelial lining of the ducts was of frequent

but not invariable occurrence. There was less dilatation of the ducts on the distal side of the ligature than one might have expected to encounter. The writer is unable to say with confidence that there was more interstitial pancreatitis or any other characteristic morbid condition of the pancreas in the animals that showed fat-necrosis than in those that did not.

In nearly all cases sections of the liver, spleen and kidneys were examined, but no alteration was discovered that appeared to have any relation to the question in hand. No tendency to hæmorrhages of a marked or constant type was noted in any of the tissues. The urine was examined for sugar in the majority of cases, and none was found.

Although the writer was less successful than Hildebrand, his work renders it evident that ligation of the pancreas in the cat may lead to fat-necrosis. It is significant that the areas of fat-necrosis were observed only in close connection with the peritoneal cavity, and especially in the neighborhood of the pancreas, which would make contact of the pancreatic juice with these areas intelligible. The circumscribed character which they usually exhibited and their occurrence at points remote from the pancreas are difficult to account for. The simultaneous existence of a streptococcus infection in cats 21, 22 and 34 is noteworthy.

Summary: The operations above described were performed on twenty animals. Well-marked fat-necrosis resulted in five; it was trifling or doubtful in three; and was absent in twelve cases.

[illegible] OF THE PANCREAS COMBINED WITH INFECTION WITH PYOGENIC BACTERIA.

In order to discover whether bacteria played any important role in the production of fat-necrosis, operations similar to those described above were afterwards undertaken, where infection with pyogenic bacteria was brought about intentionally.

Cat 37. Large, fat. The gastro-splenic portion of the pan-

creas and some of the veins issuing from it were ligated, the pancreas was lacerated with a sharp hook, and its surface was smeared with a quantity of the Staphylococcus pyogenes aureus from a fresh culture on agar. Upon the following morning the cat was found dead. The autopsy showed beginning peritonitis, and widely spread fat-necrosis in the adipose tissue near the pancreas and in the omentum, in the form of a thin, flat layer, including the most superficial cells just below the peritoneum. The Staphylococcus pyogenes aureus was recovered in cultures from the peritoneum, liver and spleen. Examination of the other viscera yielded nothing noteworthy. Microscopic sections showed well-marked beginning pancreatitis.

Cat 44. Medium-sized, fairly well-nourished. The gastro-splenic portion of the pancreas was ligated close to the duodenum, also several veins leaving it; it was lacerated with a sharp hook, and the surface was smeared with the Streptococcus pyogenes from a culture made recently from pus, on agar. On the fourth day the animal was found dead. Autopsy revealed peritonitis, especially near the pancreas. A part of the tail of the pancreas was disorganized and necrotic. There were numerous evidences of fat-necrosis in the retro-peritoneal fat of the pancreatic region and in the omentum, and a few scattered spots appeared in the mesentery and meso-rectum. Some of the areas were large and flat, others were localized. The lungs showed pneumonia and fibrinous pleurisy. Smears from the peritoneum yielded a streptococcus and a large bacillus. The streptococcus alone was recovered in cultures. The evidences of acute pancreatitis were very marked in microscopic sections.

Cat 45. Large, well-nourished. The gastro-splenic part of the pancreas was ligated; there were no laceration and no ligation of the veins, but the surface was smeared with the Staphylococcus pyogenes aureus. The animal remaining healthy in appearance was killed at the end of a week. A single small area of fat-necrosis was found close to the pancreas. Localized opacities due to thickening of the peritoneum were

seen, but there was no general peritonitis. Pancreatitis was well-marked.

The proportion of positive results was larger from these than from the first series of operations, but the number of cases was too small to permit one to draw far-reaching conclusions. The extensive fat-necrosis in cats 37 and 44 seemed as likely to have been due to the laceration of the pancreas as to the introduction of the pyogenic cocci, though the latter may have served to hasten or intensify the result.*

INTRODUCTION OF PANCREATIC TISSUE INTO ADIPOSE TISSUE.

The writer then undertook experiments with a view to testing the direct action of the excised pancreas upon fat-tissue, and finally succeeded in producing fat-necrosis in this manner. Satisfactory results were not secured immediately, however, as will be seen from the account of the first effort in this direction.

Cat 15. Large and healthy. The abdomen was opened, a piece of pancreas 20 m. m. in length just removed from another cat was fastened to the omentum with a silk ligature, and the abdomen was closed. The animal died after six days. The autopsy was made twenty four hours after death. A fibrinous exudate covered all the peritoneal surfaces. The effect of the piece of pancreas introduced upon the adjacent omentum was not clear. A small area of fat-necrosis was discovered on the surface of the left kidney, and others were seen in the omentum. Section of the animal's own pancreas showed an acute pancreatitis, apparently originating by extension inwards from the infected peritoneum, which the exposed condition of the pancreas in the cat makes possible. Fat-necrosis was seen in a large part of the fat-tissue in immediate contact with the inflamed pancreas, and the connection between the two was demonstrated in a convincing manner. Cover-glass preparations, sections and cultures showed a large bacillus and a

* [illegible] published in the "Journal of Experimental Medicine." [illegible]

small diplo- or strepto-coccus, both staining by Gram's method, in the peritoneal exudate, and in the pancreas.

In this case, the conditions seem to be practically the same as those obtained by Hlava when pancreatitis and fat-necrosis followed from the injection of the diphtheria bacillus into the pancreas. The possibility of their occurring ought to be borne in mind when pieces of pancreas or pancreatic extracts are introduced into the peritoneal cavity in experimental studies.

Owing to the danger of thus confusing the possible effects of disease of the animal's own pancreas with those of the pancreas artificially introduced, the peritoneal adipose tissue was deemed an unsuitable location, and efforts were then made to insert pieces of pancreas into the subcutaneous or intermuscular fat. As before, the cat was the animal selected. Numerous attempts to cultivate bacteria from the fresh cat's pancreas planted upon agar and kept in the incubator found it to be sterile in each case. In most of the following experiments the pancreas used was tested for its fat-splitting power, always with positive results. The locations chosen for the operations were the inguinal regions and a point a little below the sternum. The skin of the vicinity was shaved and washed with alcohol and an antiseptic solution. The instruments and silk used were sterilized with heat. The pancreas was taken fresh and with great care from the abdomen of another cat. It was inserted into the adipose tissues through a small incision, and was held in position by a silk suture, not passing through it, but uniting the edges of the tissues at the bottom of the wound. The superficial incision was closed separately with silk and collodion.

Cat 27. Thin. A piece of fresh pancreas from another cat was introduced through a small incision into the subcutaneous adipose tissue of the right inguinal region. After four days a large abscess had resulted, undoubtedly from accidental infection. The animal was killed and the abscess removed entire for histological examination. Besides the ordinary changes of suppuration, the walls of the abscess showed areas of fat-cells presenting a reddish-blue stain with hæmatoxylin and eosin

and contents having the homogeneous appearance seen in undoubted fat-necrosis. Some cells exhibited large needle-shaped crystals. Owing to the occurrence of suppuration the result was considered indecisive, but as probably indicating fat-necrosis.

Cat 30. Healthy, young animal, quite fat. Portions of fresh pancreas from another cat were introduced into the subcutaneous adipose tissue of the right inguinal region and into the adipose tissue below the sternum behind the linea alba. After four days the points of introduction had become immense abscesses. The animal was killed and both abscesses were removed entire. The histological examination gave the same result as in the last case.

Cat 33. Healthy young cat, well-nourished.. Three localities were utilized for the introduction of cat's pancreas into the adipose tissues: the middle line below the sternum, the middle line above the symphysis pubis, and the right inguinal region. After five days abscess formation had begun, so that the animal was killed and the abscesses were excised. The histological examination detected no fat-necrosis.

Cat 35. Large, well-nourished. A piece of fresh cat's pancreas was introduced below the sternum in the same manner and with the same precautions as previously, except that a portion of adipose tissue was excised to make room for the piece of pancreas. After three days an immense abscess had resulted. The animal was killed and the abscess removed. The examination of sections with the microscope gave the changes to be expected for suppuration, with alterations in the fat-cells similar to those already mentioned for cats 27 and 30.

The experiments just described seemed to show the futility of continuing along the lines followed in them. Although cultures were not made from the abscesses encountered, it was sufficiently evident that they proceeded from infection. In cases related hereafter, where identical results were obtained, the micro-organisms causing the suppuration were demonstrated. (Cats 43 and 50.) It was impossible to determine how far the changes in the tissues were to be attributed to

bacteria, and how far to the working of the pancreatic ferments. As has been remarked, infection probably took place from organisms on the skin, as the pancreas was removed with such care as to render it above suspicion. The writer has been led to suspect that the pancreas may be possessed of irritating properties which make the tissues in contact with it extremely liable to infection.

In order to observe the effects of perfectly sterile pancreas on the tissues the following technique was devised, which can be recommended when it is desired to insert solid substances hypodermically. A cannula of glass of the size and shape represented in the accompanying figure was made from ordinary glass tubing. It was sterilized in a Petri dish, and the portion of pancreas to be used was placed in the large end, with a bit of sterile black silk. The pancreas and silk were forced along to the small end with a stiff platinum wire. The skin having been shaved, cleaned and rendered aseptic as far as possible, a small incision was made through it. The adipose tissues underlying it were nicked. The small end of the cannula was then forced into the adipose tissue three or four centimetres. The pancreas and bit of silk were pushed out of it by the wire, depositing them in the desired locality, after the withdrawal of the cannula. The surface wound was closed with one or two sutures. The success of this plan was very gratifying. In seventeen experiments made according to it upon nine cats, eleven were satisfactory in excluding bacteria, while infection occurred in but four instances, and two experiments in another animal indicated doubtful infection. The black silk served admirably to identify the point of introduction.

Cat 38. Large, fat. Pieces of fresh cat's pancreas one or two millimetres in diameter were inserted into the adipose tissues just below the sternum and

in the right inguinal region, after the manner above described. On the twelfth day there had been no evidence of inflammation, and the wounds in the skin were nearly closed. The animal was killed. The points where the pancreas had been inserted were at once located by means of the black silk. Agar tubes were inoculated from both, and placed in the incubator. These tubes remained sterile. Serial sections of both regions were cut; those from below the sternum were negative; in the inguinal region a small area of fat-necrosis was found.

Cat 39. Large, fat, not very vigorous. Pieces of fresh cat's pancreas one or two millimetres in diameter were inserted through the sterile cannula into the adipose tissues of the right groin and below the sternum. The wounds of the skin healed rapidly and without incident. The animal was killed three weeks after the operation. Cultures were made from both points, which remained sterile. The examination of sections showed no fat-necrosis.

Cat 40. Small, thin. A small piece of fresh cat's pancreas was placed in the fat-tissue of the right groin through the cannula. The wound remained healthy. The cat was killed at the end of six days. Cultures from the locality remained sterile. Examination of sections did not demonstrate fat-necrosis.

Cat 42. Small, not fat. By means of the sterile cannula small bits of fresh cat's pancreas were introduced into the adipose tissue in the sternal and left inguinal regions. After the lapse of seventeen days the points in question were examined. A positive result was obtained in the left groin, where a small spot of fat-necrosis was found. (Plate V, Fig. 2.) Agar tubes from this area showed no growth. No bacteria could be found in sections stained by the Gram-Weigert method and with methylene-blue.

Cat 43. Large, fat. Through a glass cannula, bits of fresh cat's pancreas were inserted in the adipose tissues in three places: the right and left inguinal regions and the region below the sternum. At the end of three days there was much

swelling and slight fluctuation. The cat was killed. Smears were made and agar tubes inoculated from all three localities, and the spots were excised. Suppuration was evident in all, and gas-formation, as well, below the sternum. The pancreas taken in this case was from the same source as that used for Cat 42 and had produced no growth on agar. As Cat 42 had shown no suppuration, the source of infection in Cat 43 was probably the skin. The cause of the suppuration proved to be an undetermined micrococcus. Examination of sections showed probable fat-necrosis in both inguinal regions, but none in the locality below the sternum.

Cat 47. Small, sickly, not fat. Pieces of fresh pancreas of a young cat were placed in the right and left inguinal adipose tissues through the glass cannula. The cat died unexpectedly two nights later. Autopsy showed the cause of death to be bronchitis and broncho-pneumonia, probably having no relation with the operation. The edges of the wounds had parted slightly, but there was no swelling in the neighborhood, nor any of the macroscopic evidences of suppuration. Cultures from both sides yielded a small bacillus, resembling B. coli communis. The weather during this period was excessively hot, and the results of the cultures were not deemed positive evidence of ante-mortem infection of the points of introduction. On both sides, in the neighborhood of the inserted bits of pancreas and for two to three millimetres in the track taken by the cannula, the adipose tissue had a strikingly white, opaque, cheesy appearance. Examination with the microscope, fresh and in sections, demonstrated the histology of fat-necrosis.

Cat 48. Large, moderately fat. The fresh pancreas of a half-grown kitten was used. Small bits were inserted into the adipose tissues of the right and left inguinal regions through the glass cannula. At the end of five days, the cat, appearing healthy, was killed. The regions where the pancreas had been placed were not swollen; but the adipose tissues for two or three millimetres around presented a white, cheesy appearance. Examination of the cheesy matter fresh, and of the re-

PLATE V.

Fig. 1. Cat 47. Left inguinal adipose tissue, showing unaltered and necrotic fat-cells, accumulation of granules and leucocytes at point of junction, black silk, and dark fragment of tissue close to it, probably pancreas, two days after insertion of pancreas; celloidin; hæmatoxylin and eosin; × 70; photo-micrograph.

Fig. 2. Cat 42. Part of the area of fat-necrosis from the left inguinal region, seventeen days after insertion of pancreas; celloidin; hæmatoxylin and eosin; × 70; photo-micrograph.

Fig. 3. Cat 48. Left inguinal region showing isolated fragment of adipose tissue, almost wholly necrotic, and leucocytes; celloidin; hæmatoxylin and eosin. Low-power water-color sketch by Miss E. M. Porter.

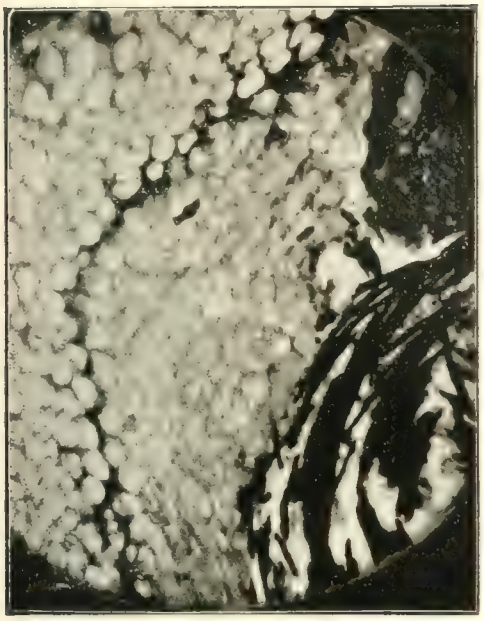


FIG. 1.

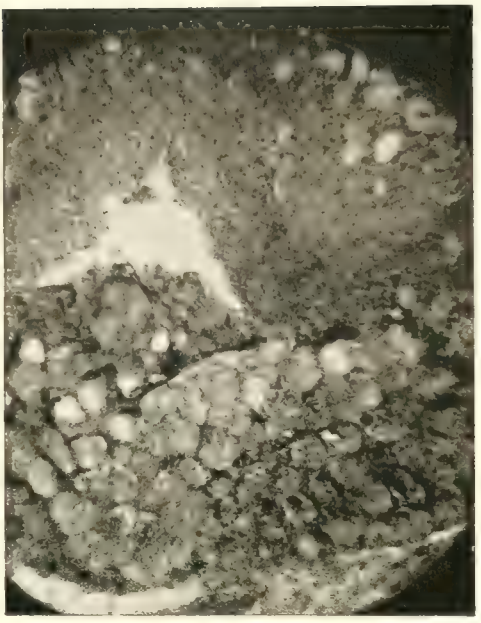


FIG. 2.

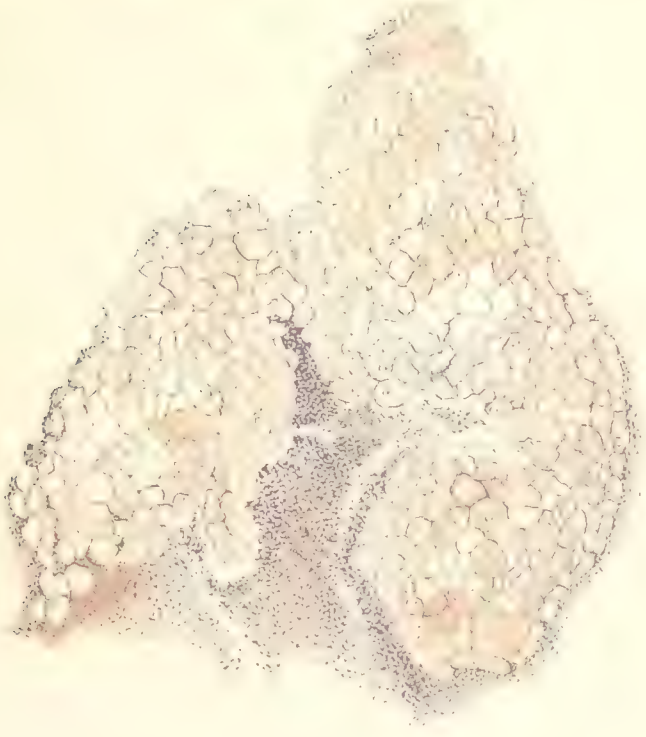


FIG. 3.

gion in sections, showed the characters of fat-necrosis. Smears and sections stained for bacteria and culture tests all gave negative results.

Cat 49. Medium-sized. Bits of fresh pancreas from a full-grown cat were introduced by means of a sterile glass cannula into the adipose tissues of both inguinal regions. After eight days, appearing healthy, the cat was killed. The skin wounds were in good condition. There was slight swelling where the pancreas had been placed, but no suppuration. There were, however, numerous minute, curd-like, white spots on the edges of the adjacent adipose tissues. Agar tubes inoculated from the region remained sterile. Smears stained for bacteria gave negative results, as did the subsequent examination of sections of the tissue for bacteria. Inspection of the curd-like masses and study of sections of the tissues gave the histology of fat-necrosis.

Cat 50. Small. Received a bit of fresh cat's pancreas in the inguinal region through the sterile glass cannula. At the end of eight days swelling and fluctuation indicated pus-formation. The pus contained an undetermined diplococcus. Nevertheless, the histological characteristics of fat-necrosis were demonstrated in fresh preparations and in hardened and stained sections.

Control Experiments.—Finally, in order to prove that the fat-necrosis was caused by the bits of pancreas inserted, and not by the injury to the adipose tissues inflicted at the operation, twelve other operations were performed on cats 52 to 55. These operations were exactly similar to those just described, including the insertion of bits of black silk, *except that the portions of pancreas were omitted.* Nothing resembling fat-necrosis appeared in any case. The tissues were examined from two to eight days after the operations. Suppuration also was always avoided, confirming the opinion, already expressed, that the presence of pancreatic ferments in the tissues renders them more liable to infection by pyogenic bacteria. Suppuration occurred after some of the operations on cats 38 to 50,

where pancreatic tissue was introduced, in spite of every precaution being taken to avoid it.

General description of the lesions at the site of introduction of the pancreas.—In those cases which resulted favorably and where the autopsies were performed only a few days after the subcutaneous insertion of the pancreas, the adipose tissues in that vicinity, which was identified by the piece of black silk, acquired a white, opaque and cheesy character.

The opacity was found to reside in certain fat-cells; while adjacent fat-cells presented a normal appearance. The altered cells exhibited a brown tinge, and their contents consisted of minute linear crystals. Ring-shaped plates were seldom seen, while they were common in the necrosis obtained in the cat after operations on the pancreas. Fragments of similar opaque material were seen which appeared to have arisen from the breaking up of such altered cells. Loose crystals and granules were also abundant.

The amount of material was too small to permit the application of micro-chemical tests in a perfectly satisfactory manner in all instances. When they could be employed the reactions were those characteristic of fat-necrosis, including sometimes the demonstration of calcium salts.

The tissues were hardened in alcohol or in formaldehyde and embedded in celloidin. Sections stained with hæmatoxylin and eosin gave satisfactory pictures. The region where the pancreas had been placed was readily discovered by means of the black silk which had been introduced with the piece of pancreas. The pancreas itself could not be identified with certainty. Fragments of disorganized tissue associated with the bits of silk, staining with eosin, not stained by the Gram-Weigert method, and exhibiting neither nuclei nor definite structure, were supposed to represent the pieces of pancreas.

The altered fat cells were found on the surface of the cavity containing the silk and these fragments of tissue (Plate V. Fig. 1). Their contents stained intensely, partly with hæmatoxylin, partly with eosin. The contents consisted in some cases of homogeneous material, in some cases of needle-shaped

or fine feathery crystals, plainly visible in balsam preparations. They often contained granules, which stained intensely with hæmatoxylin, of very variable size, usually round, but sometimes quite irregular in form. Similar granules occurred between the cells. Some of these granules may have owed their intense stain to the presence of calcium salts. Some of them appeared to have arisen from the breaking up of nuclei. Nuclei belonging to the necrotic fat-cells could not be discovered. The altered fat-cells were of normal size or slightly enlarged at most. Necrotic cells were found, which were almost completely disintegrated, and with them loose crystals and fine granular material.

Between and around the necrotic fat-cells, polynuclear leucocytes appeared, usually so as to make an extensive accumulation, though sometimes in relatively small numbers (Plate V. Fig. 3). The interstices of the neighboring unaltered adipose tissues also contained leucocytes, which, with the granules above mentioned, occasionally constituted a well-marked zone around the region of operation. Larger cells with undivided nuclei, probably endothelial, were visible in smaller numbers, especially around the fibres of silk. Fragmentation of nuclei was often seen. A fine reticulum, stained by the Gram-Weigert method, which was observed sometimes in the area of most intense necrosis, suggested the formation of fibrin. In no case was there marked evidence of hæmorrhage. Where the time after operation was longest (12 days in Cat 38, 17 days in Cat 42) the silk and area of necrotic cells were enclosed in richly cellular, new-formed fibrous tissue. In the same cases there was less accumulation of leucocytes than in the more recent ones.

It will be seen that in all essential respects the histological characters of subcutaneous adipose tissues which are subjected to the direct action of the excised pancreas may be identical with the peritoneal fat-necroses observed after ligation, laceration, and other operations on the pancreas.

Summary.—Thirteen cats were used for the subcutaneous introduction of pancreas into the adipose tissues in twenty-

four places. At nine points fat-necrosis ensued; in nine there was no fat-necrosis; in six the result was doubtful. The doubtful results were in cases in which suppuration complicated the issue. At eleven points there was no infection, two were doubtful, and eleven became infected. Selecting the eleven in which there was no infection and the two classed as doubtful, where infection was probably post-mortem, in all thirteen, we find eight points at which fat-necrosis occurred and five from which it was absent. Control experiments showed that the fat-necrosis was due to the presence of the pancreas, and not merely to the mechanical injury to the adipose tissue.

SPONTANEOUS FAT-NECROSIS IN THE CAT.

In connection with this work the writer has examined the pancreas and peritoneal adipose tissues of more than fifty cats. Quite early one was encountered exhibiting spontaneously minute white spots in the omental fat, not near the pancreas, however, which, on section, resembled closely very small areas of fat-necrosis. Later a similar condition was found in a second cat.

This animal was very fat. The abdomen was opened with a view to operating on the pancreas, when the adipose tissue of the omentum was observed to contain about ten irregular, opaque, white areas, approximately one-fourth of a millimetre in diameter. They were not observed in the vicinity of the pancreas. Two of these areas and a small bit of the pancreas were removed for examination. Sections of the pancreas exhibited nothing remarkable. One of the suspicious areas in the omental fat, under the microscope proved to be opaque; after slight pressure it broke into irregular, translucent masses, with a brown tinge, and made of fine, radiating crystals. Upon the warm stage, after the addition of glacial acetic acid, the brown masses dissolved; at the same time there was a noticeable evolution of oil droplets, apparently from the brown

masses. The small size of the suspected areas made removal beforehand of the free fat with boiling alcohol and ether impossible. Subsequent neutralization and addition of oxalic acid solution, produced an abundant precipitate of calcium oxalate crystals. Thin sections stained in hæmatoxylin showed the area partly surrounded by good-sized mononuclear cells, which, along with a small amount of fibrous tissue, partly penetrated its interior.

The condition seemed to be one of fat-necrosis arising spontaneously in the cat, and probably not recent (Plate III, Fig. 3). If it depended upon any morbid state of the pancreas, that had apparently subsided. It is possible, though not probable, that such spontaneous fat-necroses in the cat might lead to erroneous conclusions in the course of experiments like those which have already been described. The writer has endeavored to be scrupulous to avoid error in this direction.

GENERAL SUMMARY AND CONCLUSIONS.

The clinical histories of the three cases described on the first pages of this report are, in most respects, characteristic of acute pancreatitis. Fitz[7] described the symptoms of acute pancreatitis as follows: "Sudden, severe, often intense epigastric pain, without obvious cause in most cases, followed by nausea, vomiting, sensitiveness, and tympanitic swelling of the epigastrium. There is prostration, often extreme, frequent collapse, low fever, with a feeble pulse. Obstinate constipation for several days is the rule, but diarrhœa sometimes occurs."

It has been suggested that the pain and symptoms of collapse may be due to pressure upon or injury to the solar plexus. In a case of hæmorrhagic pancreatitis, Osler and Hughes[5] found the semilunar ganglia large and succulent, the ganglion-cells cloudy and indistinct, numerous small, round cells, while a group of greatly swollen Pacinian bodies lay behind the duodenum and pancreas; fat-necrosis was not recorded as present. The solar plexus and semilunar ganglion were examined with negative results in a case of Fitz's[7], (Case XXXIII.)

In Warthin's[36] case of hæmorrhagic pancreatitis, the nerve-trunks connected with the cœliac axis were involved in the necrotic process proceeding from the pancreas, but they were less affected than the other tissues of the part. Some of the nerves showed hæmorrhages. The Pacinian bodies exhibited various conditions—œdema, hæmorrhages, complete necrosis, or small cell infiltration. The semilunar ganglia were entirely necrotic. There were also evidences of chronic connective tissue proliferation about the nerve structures.

Rolleston[39] suggested that fat-necrosis might be due to some disturbance of innervation arising in the solar plexus. It is much to be regretted that no examination of the solar plexus was possible in Dr. Stockton's and the writer's Case III.

The three cases illustrate three important types of anatomical conditions under which multiple fat-necrosis occurs. In Case I, there was no pancreatitis, and the only alteration was necrosis of the acini of the pancreas. Such cases have been shown by Fitz[7] to be exceptional, (see also page 56 below). Some years ago the writer tabulated fifty-three cases of multiple fat-necrosis; all but six (11.3 per cent.), showed marked pancreatic disease, usually hæmorrhagic or gangrenous pancreatitis. Case II is an example of hæmorrhagic pancreatitis, though not of severe degree. In Case III we have a rare but very interesting condition where pancreatitis and fat-necrosis followed an injury to the pancreas, and in which the circumstances were very similar to those in the cat, where fat-necrosis was produced by operations.* The writer has never encountered a case of suppurative pancreatitis, which also appears to be a less common cause of fat-necrosis.

Bacillus coli communis was present in two of the cases. The same organism has been reported by Welch[37] and others in connection with this affection. Various other bacteria have been found by different observers. Cases have also been described in which the fat-necroses were sterile. It is probable that no organism plays more than a subordinate part in the production of fat-necrosis, although it is well known that certain bacteria possess the power of decomposing fats.[32]

Numerous observers have noted the superficial resemblance of nodules of fat-necrosis to secondary carcinomata and to tuberculous collections, which appeared in the three cases described in this report.

In all three of the cases the subjects were either stout, or, at least, the adipose tissues were well developed. The writer tabulated forty-seven cases of fat-necrosis, of which thirty-five (74.4 per cent.) were recorded as being fat, frequently very fat indeed. The subjects of this disease are not necessarily stout, however. Katz and Winkler[19] collected reports of eighty-one cases, of which thirty-five (43.2 per cent.) were

* A case of abdominal injury, with rupture of the pancreas and extensive fat-necrosis, was reported by Schmidt, Münchener Medicinische Wochenschrift, May 8, 1900.

said to be fat. It is possible that the presence of a pancreatic secretion with powerful fat-splitting properties may first lead to the accumulation of adipose tissue, and then to fat-necrosis, if, through accident or inflammation the secretion is permitted to come in contact with the adipose tissues. If this theory—which it would be very difficult to prove—be true, a certain analogy would exist between the conditions supposed and the frequent association of peptic ulcer of the stomach with a highly acid gastric juice. Speculation unsupported by actual observation shows numerous other possibilities, however: for instance, the over-developed adipose tissues of corpulent persons may contain numerous insufficiently nourished areas which may be highly susceptible to the action of pancreatic ferments or any morbid agent, or, the activity of some of the constituents of the pancreatic juice may be heightened by inflammation, though this is less probable.

The assumption just made that fat-necrosis is due to the ferment of the pancreas which decomposes fats became possible when Langerhans[21] showed that the fats in fat-necroses had been decomposed, liberating fatty acids, which usually combined again with calcium. In those rare cases in which fat-necroses have been found unassociated with disease of the pancreas and at the same time in situations remote from the abdominal cavity, it is likely that the affection is quite different in origin. The fat-necroses in bone-marrow[29] and in lipomata[6] are examples of this form; probably also the spontaneous fat-necroses which the writer has observed in the cat.

Besides the almost constant and significant association of multiple fat-necrosis with disease of the pancreas, one is impressed with the frequent appearance of *minor* forms of fat-necrosis close to the pancreas. These appear to be of two kinds—those which occurred before death, as shown by the presence of inflammatory reaction or by encapsulation, and those which may have formed post-mortem.

In the writer's experience the ante-mortem minor fat-necroses have always been associated with catarrh of the ducts or other pancreatic disease. These appear then to differ from

multiple or disseminated fat-necroses only in degree. Those cases where occlusion of the duct occurs are the simplest to understand (Case B, pancreatic calculi). Carcinoma of the pancreas, acting in the same manner, has been reported [19, 28]. Fat-necrosis has been seen also where gall-stones, which were impacted in the ampulla of Vater, might have obstructed the pancreatic duct. Larkin's case[23] seems to have been of this character. Opie[25] has collected a number of cases where similar conditions prevailed. It is possible that where multiple fat-necrosis has existed without any assignable cause (as in Case I, of Dr. Stockton and the writer), such an impacted gall-stone may have been present early and have been dislodged subsequently.

In one instance, which is considered among the examples of minor fat-necrosis (Case C), it was suspected that a hæmorrhagic pancreatitis had occurred previously. Thayer[35] and others[26] have reported cases where pancreatitis and fat-necrosis were discovered at abdominal operations and the patients recovered.

The minor fat-necroses around which no inflammatory reaction exists may have occurred post-mortem. They would then resemble the auto-digestion of the parenchyma of the pancreas described by Chiari[4]. This auto-digestion of the pancreas is also interesting in connection with the view held by many observers, that the liability of the pancreas to hæmorrhage is to be explained by the action of pancreatic ferments.

Balser[2] and others have described fat-necroses about the pancreas of the hog. The writer has found that such fat-necroses are occasionally seen in hogs in the United States.

Hildebrand[14] and others succeeded in producing fat-necroses in the cat by placing ligatures about the pancreas and its duct. Experiments are related in this report which illustrate and confirm the results of these operations. The fat-necroses may occur at points as remote from the pancreas as the mesorectum. The macroscopic appearance of the lesions, sometimes, but exceptionally, resembles the results of thrombosis or embo-

lism. The effects are always most pronounced in the immediate vicinity of the pancreas. It is possible that coincident infection by bacteria renders the tissues more liable to fat-necrosis after injury to the pancreas. Experiments in this direction were not numerous enough to be conclusive. On the other hand, there is also some ground for thinking that tissues exposed to the action of pancreatic ferments are rendered more susceptible to infection by pyogenic bacteria.

Opie[25] has recently performed the operation of ligating both pancreatic ducts in the cat, and has succeeded in producing "widespread necrosis of the fat, not only of the abdomen, but of the subcutaneous tissue and of the pericardium as well. . . . . The extent of the process is dependent upon the gradual diffusion of the fat-splitting ferment, and corresponds in some degree with the length of time which the animal survives the operation; stimulation of the secreting activity of the gland (with pilocarpin) hastens this diffusion." These experiments go far towards explaining those human cases in which fat-necrosis occurs at long distances from the pancreas, and where disease of the pancreas seems, nevertheless, to be the cause of the fat-necrosis. Formad[11] said of a remarkable case reported by him, that the whole adipose tissue of the body, not merely of the trunk, but also of the extremities, was affected with fat-necrosis. Hansemann[12] told of a patient in whom subcutaneous fat-necroses corresponded with roseola-like spots on the skin. Chiari[3] saw the subpleural, subpericardial and subcutaneous adipose tissues involved.

Katz and Winkler[19], who performed a large number of operations on the pancreas of the dog, including ligations, found a tendency on the part of the fat-necroses to appear in the immediate vicinity of the ligatures. The writer observed the same relation in some cases. Katz and Winkler frequently found hæmorrhages in and about the pancreas.

Flexner[9] succeeded in demonstrating a fat-splitting ferment in fat-necroses by chemical tests, both in human subjects and in lesions produced in cats. His results have been confirmed by Opie[25].

The direct action of a suspension of pancreas on adipose tissue was first tested by Langerhans[22], who produced fat-necrosis in one instance. This experiment has been repeated by the writer in a modified form. The direct introduction of fresh cat's pancreas into subcutaneous adipose tissue produced fat-necrosis in the vicinity in about half of the cases. This happened also when it was proved by bacteriological examination that no accidental infection had occurred. It was shown by control experiments that the fat-necrosis did not result merely from injury to the adipose tissue when pancreatic ferments were not present. Oser and Katz[19] secured about the same results. Opie[25] succeeded in turning the ligated ends of the pancreatic ducts into the subcutaneous tissue; fat-necrosis in this situation resulted.

Hlava[15] produced pancreatitis and fat-necrosis in the cat by injecting the diphtheria bacillus into the pancreas. The writer observed a similar result from an accidental infection of the cat's pancreas with a streptococcus. Hlava[16] and Flexner[10] have shown that pancreatitis and fat-necrosis may be produced in animals by the injection of hydrochloric acid and other irritants into the pancreatic duct. Hlava found some probability in the theory that pancreatitis might result from the entrance of gastric juice into the pancreatic duct.

Whitney[38] called attention to the resemblance of fat-necroses to the localized necroses produced in the viscera by bacterial and other poisons. The focal character which fat-necroses often show is difficult to explain by the action of a soluble ferment. More exact knowledge of the nature of the fat-splitting ferment of the pancreas, and of the causation of hæmorrhagic pancreatitis, and further studies on the condition of the solar plexus in pancreatitis, are much to be desired.

BIBLIOGRAPHY.

[1] Balser. Virchow's Arch. f. path. Anat. etc. 1882. Bd. XC. p. 520.

[2] Balser. Verhandl. d. Cong. f. innere Med. Wiesb. 1892, also Wien. Med. W[illegible] 1892 [illegible]

[3] Chiari. Prager Medicinische Wochenschrift 1883, p. 299.

[4] Chiari. Zeitschr. f. Heilkunde. 1896. Bd. XVII, p. 69.

[illegible] Inaug. Diss. Göttingen 1895.

[illegible] Arch. d. Int. de Méd. exp. 1888. Bd. XXXII, p. 710.

[7] Fitz. Boston Med. and Surg. Jour. 1889. Vol. CXX, p. 181.

[8] Fitz. Boston Med. and Surg. Jour. 1892. Vol. CXXVII, p. 571.

[9] Flexner. Jour. Experimental Med. 1897. Vol. II, p. 413.

[10] Flexner. Contributions to the Science of Medicine, twenty-fifth Anniversary. W. H. W[illegible] 1900, p. 743.

[11] Formad. University Med. Mag. 1891-92. Vol. IV, p. 45.

[12] Hansemann. Berl. Klin. Wochen. 1889. Jahrg. XXVI, p. 1115.

[13] [illegible] Centralbl. f. Chirurg. 1895. Bd. XXII, p. 257.

[14] Hildebrand. Arch. f. Klin. Chirurg. 1898. Bd. LVII, p. 438.

[15] Hlava. Sborn. lék. v. Praze (Archives bohémes de Médicine.) 1893. Vol. IV, p. [illegible]

[16] Hlava. Bull. internat. de l'Acad. des Sciences de Bohéme. 1898.

[17] Jung. Inaug. Diss. Göttingen. 1895, also Centralbl. f. Chirurg. 1895. Bd. XXII, [illegible]

[18] Kasahara. Virchow's Arch. f. path. Anat. etc. 1896. Bd. CXLIII, p. 111.

[illegible] Die multiple Fettgewebsnekrose. Berlin 1900.

[illegible] Arch. f. klin. Chirurg. 1900. Bd. XLVIII, p. 771.

[21] Langerhans. Virchow's Arch. f. path. Anat. etc. 1890. Bd. CXXII, p. [illegible]

[22] Langerhans. Festschritt. R. Virchow. Berlin 1891.

[23] Larkin. Proc. N. Y. Path. Soc. 1897-98, p. 98.

[24] Nimier. Revue de Med. 1894. T. XIV, p. 353.

[25] Opie. Contributions to the Science of Medicine, twenty-fifth Anniversary. W. H. Welch. 1900. p. 859.

[26] Osler. Practice of Medicine. 3d ed. 1899. p. 590.

[27] Osler and Hughes. Trans. Path. Soc. Phila. 1887. Vol XIII, p. 24.

[28] Pitt. Trans. Path. Soc. London. 1894. Vol. XLV, p. 91.

[29] Ponfick. Virchow's Arch. f. path. Anat. etc. 1872. Bd. LVI, p. 541.

[30] Rolleston. Trans. Path. Soc. London, 1893. Vol. XLIV, p. 71.

[31] Sarfert. Centralbl. f. Chirurg. 1895. Bd. XXII, p. 652.

[32] Sommaruga. Zeitschr. f. Hygiene. 1894. Bd. XVIII, p. 491.

[33] Stockton and Williams. Amer Jour. Med. Sci. 1895. Vol. CX, p. 251.

[34] Stockton and Williams. Phila. Med. Jour. 1900. Vol. VI.

[35] Thayer. Amer. Jour. Med. Sci. 1895. Vol. CX, p. 396.

[36] Warthin. Phila. Med. Jour. 1898. Vol. II, p. 1067.

[37] Welch. Medical News. 1891. Vol. LIX, p. 669.

[38] Whitney. Boston Med. and Surg. Jour. 1895. Vol. CXXXII, p. 361.

[39] Williams. Boston Med. and Surg. Jour. 1897. Vol. CXXXVI, p. 345.

[40] Williams. Jour. Experimental Med. 1898. Vol. III, p. 585.